THE AMERICAN JOURNAL OF PATHOLOGY

VOLUME XXVIII JULY–AUGUST, 1952 NUMBER 4

THE CHOLESTEROL OF HYALINE ARTERIOLOSCLEROSIS *

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The more frequent occurrence of hyaline changes in the arterioles of the kidney in hypertensive persons than in those without hypertension has been generally accepted. The studies reported in this paper were inaugurated for the purpose of determining the nature of the hyaline material by histochemical means, and its relationships to atheromatosis of larger arteries and to hypertension.

Histochemical study of the hyaline material of renal arteriolosclerosis discloses both lipid and non-lipid content.[1] The lipid part absorbs sudanophilic dyes readily, such as Sudan IV.[2] The Schultz reaction for sterols gives the characteristic greenish reaction, indicating a cholesterol content of the hyaline regions of renal arteriolosclerosis.[1]

We have applied the Schultz modification of the Liebermann-Burchard test for cholesterol to the kidney in 47 cases of hyaline arteriolosclerosis and in 20 control cases. The test has been applied also in correlative histochemical studies to extrarenal arterioles, to arteries, and to other tissues of the body.

MATERIALS AND METHODS

The method of Schultz for cholesterol, based on the Liebermann-Burchard reaction, was applied as follows.[3] Frozen sections of formalin-fixed tissue, cut at approximately 15 μ, were placed in a mordant of 2.5 per cent aqueous solution of ferric alum for 3 days at room temperature. A section was rinsed in distilled water and blotted dry on a slide. It was then treated with the Schultz reagent, which consisted of equal parts of concentrated sulfuric acid and glacial acetic acid. The reagent was applied to the section by a glass rod, a coverslip

* This investigation was supported (in part) by a research grant from the National Heart Institute, U.S. Public Health Service. Publication of the color plate has been made possible by a grant from the Jefferson County Chapter of the Alabama Heart Association.

Presented at the Forty-eighth Annual Meeting of the American Association of Pathologists and Bacteriologists, Cleveland, April 26, 1951.

Received for publication, October 12, 1951.

was added, and microscopic study was made. The green color of a positive reaction appeared in a minute or so and lasted about an hour. The formation of air bubbles could be controlled somewhat by careful blotting of the tissue and by use of H_2SO_4 of at least 98 per cent purity. The strong acid altered the tissues but structures could be recognized by cutting down the light. No counterstain was applied. Since preparations were not permanent, photographs were taken for record.

Forty-seven cases with hyaline arterioles, and 20 control cases were tested with the Schultz reagent.

Selection of cases of renal arteriolosclerosis was made by examination of a single, routine, hematoxylin and eosin stained, paraffin section from each of many necropsies until 47 cases were located which exhibited hyaline arterioles. These were found in persons between 20 and 80 years of age. Accordingly, control cases devoid of hyaline arterioles in routine sections were selected from the same age period. Selection was made by an examiner unaware of other features of the case.

Correlative studies were made of many other organs and tissues.

Results

Of the 47 cases with hyaline arterioles in paraffin sections, Schultz-positive arterioles were identified in 43. Sudanophilic arterioles were demonstrated in 46 cases.

Of the 20 control cases with no hyaline arterioles in a routine hematoxylin and eosin section, Schultz-positive arterioles were demonstrated in 2, Schultz-negative arterioles in 14, while arterioles were not identified in similar preparations in 4. Sudanophilic arterioles were demonstrated in 4 of the 20 cases.

Correlative studies on one of the cases of arteriolosclerosis showed hyaline arterioles to be Schultz-positive in spleen, pancreas, liver, adrenal glands, and striated muscle. These arterioles were sudanophilic also. No hyaline arterioles were found in cardiac muscle, testes, skin, brain, and adipose tissue, and the Schultz test and Sudan staining also were negative in these arterioles.

The atheromas of arteries were consistently Schultz-positive (Fig. 6). These were demonstrated in aorta, common carotid, cerebral, splenic, and coronary arteries. The atheromas were sudanophilic but not hyaline.

Other tissues showed Schultz-positive lipid material in the adrenal cortex, in myelin of the nervous system, and in several other sites.

Concerning the relationship of hyaline arteriolosclerosis of the kid-

ney to hypertension, additional information was gathered. In 46 of the 47 cases the heart weighed more than 300 gm. In 16 of the 20 control cases the heart weighed more than 300 gm. The average of the 47 hearts was 497 gm., and of the 20 control hearts, 381 gm. Thus there was a tendency toward a greater degree of cardiac hypertrophy in the cases with hyaline arterioles in the kidney.

While hypertrophy of the heart in the absence of valvular disease is not considered by all to be an index of hypertension, many would agree that this increased weight of heart was suggestive evidence that hypertension had been present. It was impossible in many cases to say whether or not hypertension had been present during life, since many

TABLE I
Age of Cases Selected for Study of Cholesterol in Arterioles

Decade	47 cases	20 controls	Decade	47 cases	20 controls
Third	2	2	Sixth	6	
Fourth	6	4	Seventh	14	4
Fifth	10	4	Eighth	9	6

patients were in circulatory failure and had not been in the hospital long. However, 34 of the 47, or 72 per cent, had shown diastolic pressures over 100, while only 2 of the 20 controls, or 10 per cent, had shown diastolic pressures over 100. These data suggest a relationship between renal hyaline arteriosclerosis and hypertension.

Hyaline arteriolosclerosis did not appear to be merely a function of age. It occurred in the third and fourth decades and yet failed, at times, to occur in the eighth decade among the controls (Table I).

DISCUSSION

The Specificity of the Schultz Test

While Schultz[3] described his method for cholesterol in tissues in 1924, it has not been as widely used as it deserves. Knouff, Brown, and Schneider,[4] in 1941, used one adrenal gland of a guinea-pig for histochemical and the other for chemical examination and concluded that the Schultz histochemical test appeared adequate for distinguishing wide variations in the cholesterol content of the adrenal gland. It is usually stated that color reactions of this type are characteristic of steroids which possess some degree of unsaturation. Everett[5] wrote (1947): "The substances which give the characteristic color response in the Schultz test are evidently limited to the diols such as are formed from cholesterol by mild oxidation procedures." Cholesterol is the

characteristic sterol of higher animals and occurs in largest amounts in brain, adrenal glands, and egg yolk.

In our correlative histochemical studies with the Schultz reaction we found adrenal cortex and myelin of the central nervous system to give vivid color response. This corresponds with the chemical results. Most cells of the body, however, did not give a positive Schultz reaction, even though it is generally stated that all cells have cholesterol in them. This may indicate that the general statement is untrue, or that the test is not sensitive to minute amounts, or that the cholesterol is bound to other substances so as to prevent a positive test.

Among pathologic lesions, xanthomas, atheromas of larger arteries, and macrophages of cerebral infarcts gave positive Schultz staining and were sudanophilic also. Extrarenal hyaline arterioles, encountered in generalized arteriolosclerosis, were usually Schultz-positive and sudanophilic.

Sudanophilia and Cholesterol Test

The absorption of Sudan IV by fatty substances indicates only fatty or oily nature. As might be expected, therefore, several tissues were sudanophilic, but not Schultz-positive. This was true of adipose tissue, sebaceous glands, the lipid of fatty change of liver, lipochrome of heart muscle and of ganglion cells. Substances which were Schultz-positive were usually sudanophilic. This was true of adrenal cortex, atheromas, and hyaline arterioles. Myelin of the central nervous system, however, gave a strongly positive cholesterol test, but was not sudanophilic. With respect to the cholesterol of hyaline arterioles, sudanophilia appears to run closely parallel to the positive cholesterol test, and it is probable that generalizations concerning sudanophilia of the arterioles would likewise apply to a positive cholesterol test on them.

Wilens and Elster[2] (February, 1950) concluded that sudanophilic lipid deposition in the walls of renal arterioles occurs as commonly as it does in the intima of large arteries, and that its incidence is significantly increased in hypertension and in diabetes. Our studies add the information that the lipid substance of the hyaline arterioles gives a positive Schultz test for cholesterol, as do the atheromas of larger arteries. In a publication of May, 1950, Baker and Kent[1] presented evidence that the hyalin of arteriolosclerosis may have components other than lipid and other than cholesterol.

The Significance of the Cholesterol Deposits in Arterioles

Cholesterol deposits in arterioles may have the same significance as such deposits in larger arteries. It is possible that the lipid is imbibed

from the blood stream directly and that the increased incidence in hypertension and diabetes represents a driving of blood lipid into the wall. Such a mechanism would be even more reasonable if applied to the arterioles than if applied to the arteries because the thin arteriolar wall is in closer contact with the blood. On the other hand, it has not been shown clearly, as yet, that lipid change in vessel walls may not be due to local changes of a degenerative or regressive nature, perhaps like the fatty change of liver or heart muscle.

The change in the arterioles is like that of the arteries in that the lipids are apparently chiefly cholesterol or cholesterol esters in the two regions. In the arteriolar lipidosis, however, there is no tendency for the cholesterol to occur in crystals or in macrophages. Nor is there any apparent tendency for calcium to be deposited in conjunction with the lipid. This may be merely a matter of the smaller amount of material in the arteriolar wall, and the crystals and lipophages in the larger masses of atheromas may be secondarily derived from imbibed cholesterol. In one other respect there is dissimilarity, for the lipid-containing arteriole stains regularly like hyalin in hematoxylin and eosin sections while the atheromas do not exhibit this feature impressively.

Perhaps the bulk of evidence suggests that arteriolar lipidosis is the result of hypertension. Can we say definitely, however, that arteriolar lipidosis, by producing stenosis of arterioles, may not be a cause of hypertension?

Relation of Age, Sex, and Hypertension to Cholesterol Deposits in Arterioles

It is probable that the same conclusions can be drawn with respect to cholesterol staining as to sudanophilia of these arterioles. Wilens and Elster,[2] in a thorough study of the factors governing sudanophilia of arterioles, concluded that sudanophilia is relatively infrequent and slight in the very young and increases in incidence slowly but progressively with advancing age in non-hypertensive persons; that it is somewhat more common in hypertensive or diabetic women than in similar groups of men; and that there is no difference in sex incidence of renal arteriolar lipidosis in non-hypertensive persons or in whites and Negroes.

Further, these authors[2] found that the incidence of renal arteriolar lipidosis is significantly increased in all forms of hypertension except that associated with chronic glomerulonephritis; that it is significantly increased in diabetes; that it is not increased in renal diseases that are not associated with hypertension. They found, also, that the incidence

and severity of renal arteriolar lipidosis is increased in the presence of arteriolar sclerosis, although scanty lipid deposits are frequently found in arterioles that are otherwise unaltered.

Summary and Conclusions

Hyaline arterioles of the kidney were found to contain, in 43 of 47 cases, a lipid material which, on the basis of the Schultz modification of the Liebermann-Burchard test, was cholesterol.

Control studies on 20 cases indicated that in the absence of hyaline arterioles, in a single routine section of kidney from the necropsy, lipid in general and cholesterol in particular were less frequently demonstrated in the arteriolar walls.

Correlative studies showed the cholesterol test to be positive for hyaline arterioles of other organs, for atheromas of larger arteries, and for adrenal cortex and myelin. Adipose tissue and lipochrome failed to give a positive cholesterol test, although they were sudanophilic. Conversely, myelin gave a positive cholesterol test, though it was not sudanophilic. The positive cholesterol test in hyaline arterioles correlated well with the sudanophilia of these arterioles.

Since the hyalin of renal arteriolosclerosis contains cholesterol, like atheromas of larger arteries, a similarity of arteriolosclerosis to atheromatosis is indicated.

The hyaline arteriolosclerosis in hypertensive persons is accompanied by cholesterol deposition, on the basis of the Schultz test, more frequently than in non-hypertensive persons.

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[*Illustrations follow*]

DESCRIPTION OF PLATE

PLATE 81

FIG. 1. Hyaline arteriolosclerosis of kidney. Paraffin section. Hematoxylin and eosin stain. The hyaline, eosin-staining regions give a positive Schultz[3] test for cholesterol in appropriately treated frozen sections, more frequently in hypertensive than in non-hypertensive persons. × 103.

FIG. 2. Hyaline arteriolosclerosis of kidney. Frozen section. Sudan IV and hematoxylin stains. The sudanophilia indicates the fatty nature of the hyaline region, but is less specific chemically than the Schultz test. × 227.

FIG. 3. Hyaline arteriolosclerosis of kidney. Frozen section. Schultz modification of the Liebermann-Burchard reaction for cholesterol. No counterstain. The green positive reaction indicates cholesterol in the arteriolar wall, on the basis of this reaction. × 94.

FIG. 4. Hyaline arteriolosclerosis of spleen. Frozen section. Schultz modification of the Liebermann-Burchard reaction for cholesterol. No counterstain. × 94.

FIG. 5. Hyaline arteriolosclerosis of pancreas. Frozen section. Schultz modification of the Liebermann-Burchard reaction for cholesterol. No counterstain. × 97.

FIG. 6. Arteriosclerosis of aorta. Frozen section. Schultz modification of the Liebermann-Burchard reaction for cholesterol. No counterstain. The cholesterol test is positive in atheromata of arteries as well as in the hyaline regions of the arterioles. Chemical tests have shown the high cholesterol and cholesterol-ester content of atheromata of arteries. Presumably, chemical tests would show the same for arterioles, on the basis of the histochemical studies reported herewith. × 14.3.

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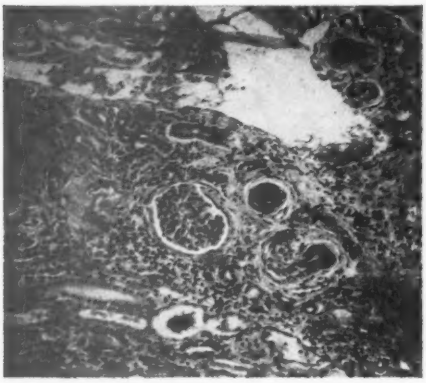

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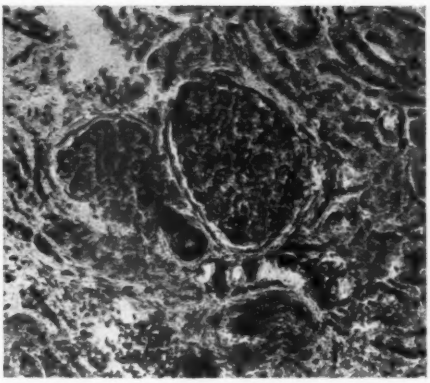

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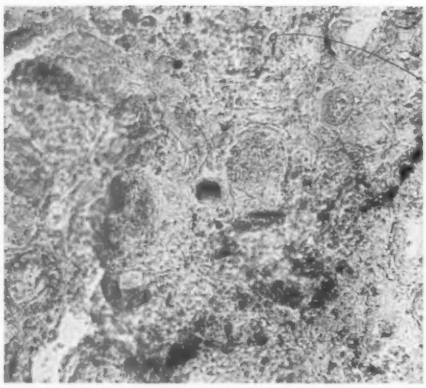

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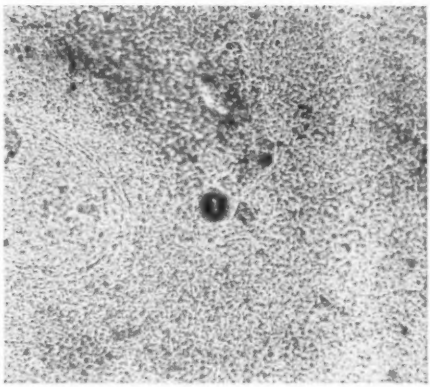

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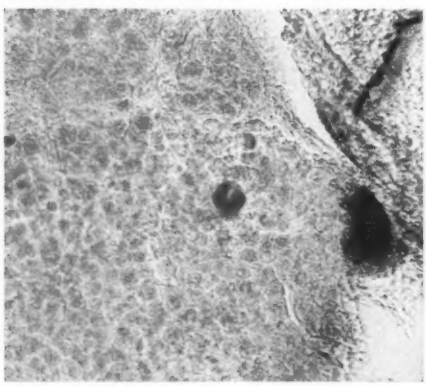

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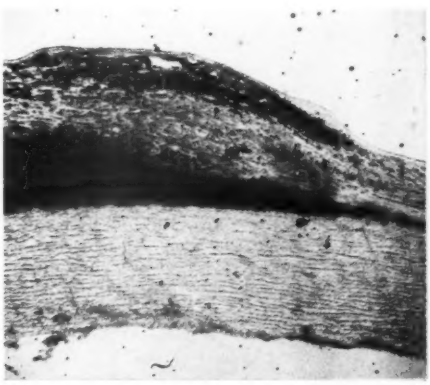

Baker and Selikoff

Cholesterol of Hyaline Arteriolosclerosis

THE HISTOPATHOLOGY OF COXSACKIE VIRUS INFECTION IN MICE

II. Histochemical Observations on the Lesions in Muscle and Fat*

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Following injection of newborn mice with certain antigenic types of Coxsackie virus (*viz.*, Connecticut-5), lesions regularly occur in the striated muscle, fat bodies, central nervous system, myocardium, pancreas, and liver. The morphologic features of these lesions have been detailed in a previous communication[1] and their relation to virus growth and distribution, with particular reference to the striated muscle, has been studied.[2] The observations presented in this paper were undertaken to explain certain of the structural or tinctorial appearances which have been described for the lesions in striated muscle and adipose tissue, or to demonstrate some of the chemical properties of the infected muscle or fat bodies, using available histochemical technics.

Materials and Methods

Groups of mice were inoculated on the first day of life with Conn.-5, Ohio-1, Texas-1, or Nancy strains of Coxsackie virus, according to the methods previously given.[1,2] Each of these strains represents a distinct antigenic prototype.[3] Tissues were collected on the first day of paralysis, and subsequently as they became available on the second, third, fifth, and seventh days of survival, after the first signs of disease. These were prepared as will be described for investigation of basic dye affinity, mineral content, nucleic acids, lipids, birefringence, phosphatase, and vital staining.

Birefringence was studied in frozen sections of unfixed and formalin-fixed material, unstained or stained with Sudan black.

Ferric iron was determined by the Prussian blue method (as recommended by Lison), with a prolonged exposure.[4] Ferrous iron was detected by 20 per cent potassium ferricyanide and 1 per cent hydrochloric acid without pre-treatment by ammonium sulfide. Ten per cent formalin buffered at pH 7 was the fixative used for determination of minerals (iron, calcium, phosphate).[5] The presence of inorganic cal-

* Aided by grants from the National Cancer Institute of the United States Public Health Service and the National Foundation for Infantile Paralysis.
Received for publication, November 23, 1951.

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cium was determined by the formation of gypsum crystals from the locus in question upon the addition of dilute sulfuric acid (5 per cent).[6] Gallamine blue forms blue or purple lakes with iron and with calcium.[7] It has been employed in these studies simply to detect the presence of mineralized foci.

The presence of carbonate was sought by the evolution of gas from the particular focus in question upon the addition of dilute (5 per cent) sulfuric acid.

Inorganic phosphate was detected by the molybdenum blue method,[8] slightly modified from Feigl[9] and Serra and Queiroz-Lopes.[10]

Basophilia was determined by staining for 1 to 2 hours with aqueous methylene blue 10^{-4} M buffered at pH 7.6, 6.6, and 5.7. The slides were washed briefly in water and air dried before being cleared in xylol. Formalin was the fixative employed.

Trypan blue was used to detect necrotic cells by its well known ability to stain the cytoplasm and nuclei of such cells diffusely. An aqueous solution (0.05 cc. of a 2 per cent solution per 1 gm. of body weight) was injected subcutaneously into the mice which were sacrificed 4 to 6 hours later. The organs were fixed in Helly's fluid for 4 to 18 hours and thin sections (1 to 2 mm. thick) run up rapidly according to the method of Forkner.[11] The sections were lightly counterstained with paracarmine.

The Feulgen reaction for desoxyribonucleic acid was carried out by exposing sections to Schiff's reagent (fuchsin sulfurous acid) after hydrolysis in normal hydrochloric acid at 60° C. for intervals of from 10 to 60 minutes. Control sections were treated similarly except that water was substituted for hydrochloric acid. Details of the method may be found in Lison[12] and Stowell.[13] Formalin was used as the fixative.

Ribonucleic acid was determined by subjecting the sections from formalin-fixed tissue to the action of ribonuclease (Armour). This was prepared according to the method of Kunitz, and with the proteolytic activity destroyed, as shown by McDonald.[14] The enzyme was made up to a concentration of 6 mg. per 20 ml. of distilled water. The pH of such solutions was approximately 6.0 to 6.8. The test section and another which had been simply incubated in water at the same temperature and time (57 to 59° C. for 2 hours) were then stained progressively with methylene blue 10^{-4} M buffered at pH 7.6. The regions of basophilia that had been removed by the enzyme were considered to be sites of ribonucleic acid.

Lipids were determined by several methods. Sudan IV and Sudan black in glycol[15] were used to stain frozen sections prepared from tis-

sue fixed in 10 per cent formalin buffered at pH 7. The presence of crystalline lipids was determined by use of the polarizing microscope before and after extraction of the frozen sections with cold acetone for 30 minutes.

Phosphatase activity was determined by the Gomori technics[16] with the precautions established by Goetsch and Reynolds[17] for acid phosphatase.

Muscle

Murine Coxsackie virus disease, brought about by the strains of virus used in the present investigation, is characterized by a necrotizing myopathy in which there is more or less widespread segmental injury of the muscle fibers, resembling Zenker's degeneration. In the degradation of the affected muscle segments, successive stages are recognized as leading to ultimate hyalinization. The most important of these are a proliferation of muscle nuclei, which ultimately disintegrate, and an alteration of the myofibrils. These come to appear as coarse dark ("basophilic") longitudinal striae, prior to their ultimate disappearance. Regeneration begins even before inflammatory reaction, and becomes increasingly prominent through the ingrowth of slender sarcoblastic slips as phagocytic debridement takes place. These new plasmodial elements have central rows of nuclei with rod-like nucleoli and basophilic cytoplasm, and differentiate into young mature muscle fibers in the course of 7 to 10 days, leading to restoration of the muscle in surviving specimens.

Degeneration

Birefringence. Observations were made of birefringence on (1) apparently normal striated fibers, (2) affected segments with early changes which still showed coarse longitudinal striae, (3) segments with more advanced changes, but in which some longitudinal striation was still evident, and (4) completely homogeneous hyalinized segments. The results of these observations are summarized in Table I, in which the degree of birefringence as judged by direct visual observation is ex-

Table I

Degree of Birefringence at Various Stages of Degeneration as Compared to Normal Muscle Fibers

Uninvolved fibers as control	Early degeneration (striae present)	Intermediate stages (remnants of striae)	Hyaline segments
++ to +++	30* ++ to ++++	16 ++ to +++	16 + to ++
	20 0 to +	25 0 to +	25 0
	50	41	41

* The numerals give the number of segments evaluated.

pressed from 1 plus, indicating least, to 4 plus, greatest. In each case, control observations on normal fibers disclosed the usual bright, doubly refractile A disks, compared with which the birefringence of the early degenerated segments was more marked. The birefringence of the uninvolved fibers used as a control was estimated at 2 or 3 plus.

The birefringence was manifested as a diffuse glow (Fig. 4), or in the form of bright longitudinal bands, often at the periphery, and apparently corresponding with the longitudinal striae in most cases. The sign of the birefringence was positive, as determined using the first order red plate, indicating a submicroscopic fibrillar structure, according to the theory of Wiener.[18] No cross striations were visualized with the polarizing microscope in such altered segments, nor could cross striations be identified in similarly altered segments in sections stained with Mallory's phosphotungstic acid hematoxylin. The results tend to show that birefringence, which depends upon a definite orientation of submicroscopic elements, becomes accentuated and then uniform along the fiber length with the onset of degenerative changes. This quality is gradually lost as all visible structure disappears from the segment. From this it may be inferred that hyalinization involves a disorganization of submicroscopic fibrillar structure.

The positive double refraction of striated muscle is assumed to be due to oriented myosin filaments. It is known that the myosin-containing myofibrils extend continuously through the sarcomere.[19] It has been postulated by Dempsey, Wislocki, and Singer[20] that the difference in optical behavior of the anisotropic and isotropic segments resides in the presence of negatively birefringent phospholipid in isotropic segments, owing to the perpendicular orientation of these lipid molecules with respect to the positively birefringent myosin filaments.

Weber,[21] however, explained the lack of birefringence in the I disk by supposing that in this segment the parallel-fibered micelles consist of the strongly negatively birefringent "N-protein," the positive birefringence being compensated by its own negative birefringence. The studies reported by Matoltsy[22] support the view that the optical properties of the isotropic segment are due to "N-protein."

Basophilia. The described affinity of many of the degenerating muscle segments for hematoxylin and for certain basic dyes led to an examination of the actual degree of basophilia of the various altered muscle segments as determined under controlled conditions. In sections stained with methylene blue, as indicated in Methods, there were evident differences in tinctorial affinity between the degenerating segments and the still normal striated fibers. In many instances the longitudinal striae of the early stages of degeneration were found to be

strongly stained. The more structurally homogeneous segments of the intermediate stage had a marked affinity for the basic dye, and were more strongly stained than adjacent unaffected fibers. The distribution of density of staining within the segment was often not uniform. The disintegrated segments of the late stages of necrosis had little attraction for methylene blue or, indeed, for most other stains. The persistent residual masses sometimes found in otherwise regenerated muscle retained a striking degree of basophilia.

Nucleic Acid. That the diffuse basophilia of the early degenerating segments was not due to either desoxyribose or ribose nucleic acids was demonstrated by consistently negative Feulgen reactions, and by the unalterability of their staining capacity with methylene blue (as compared with controls) after ribonuclease digestion. The Feulgen reaction, however, demonstrated the nuclear increase and fragmentation in the earliest stages of degeneration. Muscle segments in all stages of degradation, as well as residual lesions, were available for these tests.

Phosphate and Phosphatase. Application to sections of the procedures for either acid or alkaline phosphatase resulted in heavy metallic precipitates on many of the degenerated fiber segments. But a brown-black coloration was present also in control slides. The densest deposits in all cases were present in those segments showing intermediate stages of injury: in many of these segments the longitudinal striae were colored densely black, against a dark brown background. In other instances, the segment was uniformly black or brown. However, staining of necrotic segments seemed to be irregular or capricious, since many obviously hyalinized segments were only faintly stained. The faintly stained masses were those devoid of visible structure, and it would appear that the heaviest deposits occurred not only in those segments with longitudinal striae but in or on the striae themselves (Fig. 2).

Since precipitate forms in these segments without prior exposure to substrate, it was assumed to be due to organically bound ionizable phosphates or to other acidic groups by which the heavy metal was precipitated and that the actual phosphatase activity could not be evaluated.

Histochemical examination for ionizable phosphate using a modified molybdate method revealed intense coloration localized in some but not all of the degenerated segments. The distribution of intensely phosphate-positive segments was similar to that observed in the phosphatase preparations. Segments in which there were strial remnants appeared most strongly positive, the striae themselves sometimes stand-

ing out as more intensely stained. The apparently homogeneous segments were colored irregularly or not at all. In order to avoid possible adsorption of phosphate that might diffuse from the neighboring developing bone, the muscle was dissected away from bone prior to fixation in some instances, and on the slide after sectioning in other cases, before the phosphate procedure was carried out (Fig. 5).

Lipids. In sections of normal fibers stained with Sudan black the I disks are dark gray and the A disks relatively unstained. The general background of the segments in the intermediate and later phases of degeneration was stained irregularly faint gray, indicating a dispersal of the sudanophilic lipids. In many segments still possessing visible longitudinal striae or myofibrils, fine jet black droplets and globules were aligned in rows of varying size between the striae; such appearances were thought indicative of fatty changes occurring early in the period of injury or with less severe injury.

Trypan Blue, Intravital. The degenerating muscle segments of intravitally stained infected mice were found perfectly demarcated from the unstained intact muscle, permitting accurate study of their distribution, forms, and lengths. The selective scattered distribution of lesions within particular muscles was very clearly demonstrated in the animals infected with the Conn.-5 strain. The diffuse staining of dead or injured cells, including the nuclei, by trypan blue has long been known as a technic permitting very precise distinction. The use of intravital staining with trypan blue to follow the inflammatory and regenerative sequences in injured skeletal muscle has had its most elaborate treatment in the studies of Pfuhl.[23] In the virus-produced murine myopathy, segments showing the earliest or mildest changes were found to have slightly stained backgrounds and groups of more darkly stained cross striations; a few of the increased number of nuclei usually present in such segments were deeply dyed (*i.e.*, dead or dying). In older, more severely affected segments, there was darker, more homogeneously colored content, and the proliferated distorted nuclei were always densely stained. Such appearances have been called "dissociative degeneration" by Pfuhl, who believed that the fibrils degenerate before the nuclei and sarcoplasm in a slower or milder sequence than the contrasting "true" waxy degeneration, in which all elements are destroyed simultaneously. In our material, they would appear to be different stages leading to the same end.

Minerals. The pronounced selective hematoxylin staining of the longitudinal striae of many segments in an intermediate stage of degradation suggests mineralization of these foci. Ferric and ferrous iron were localized identically on the longitudinal striae (altered myo-

fibrils) in degenerating muscle segments. In such segments, the striae were delineated by the stains for iron and more precisely by the gallamine blue method,[7] while the surrounding sarcoplasm was practically uncolored (Fig. 6). These fibers gave irregular and inconclusive results for calcium; with alizarin the coloration was only suggestive of the formation of calcium lakes, but the more definitive microchemical test depending upon gypsum formation was not confirmatory of the presence of significant quantities of calcium.

As early as the first day of apparent disease, heavily and rather uniformly mineralized segments may be found in some specimens. In these, deposits of ferrous and ferric iron were detectable; the necrotic fibers gave negative results when tested for carbonates.

Interpretation of Histochemical Changes in Hyaline Degeneration of Muscle

The affinity for basic dyes of the altered striated muscle fibers in such conditions as "waxy degeneration," trichinosis, and other regressive conditions has been recognized casually.[24,25] It has been cited, together with evidence from *in vitro* experiments, in support of the theory that lactic acid accumulates in injured muscle, and that this lactic acid is, in fact, the common causative agent of hyaline or waxy (Zenker's) degeneration of muscle.[25] This concept of the etiologic rôle of lactic acid has not been substantiated in subsequent muscle-fatigue experiments.[26] In itself, the basophilia of the altered segments is hardly credible evidence for the presence of lactic acid or the formation of "acid proteins" by it. Hydrogen ions from lactic acid are in fact normally bound by some as yet unidentified protein in the physiologic energy-producing transactions of the muscle, in its work-phase.[21]

The nature of the basophilic material of the A disks of normal striated muscle is not known. Dempsey *et al.*,[20] on the basis of its binding capacity for methylene blue over a wide pH range, found that its dissociation is weaker than that of the phosphate or sulfate groups of nucleoproteins or mucoproteins respectively, and suggested that muscle fibers contain a different acid grouping.

The basophilia of the fiber segments with early degeneration studied in the present investigation is due neither to nucleoprotein nor to a metachromatic substance, but may be correlated with the presence of hydrolyzable, ionizable phosphate which is locally bound. The origin of the high concentration of phosphate in the degenerated segments, and the substances with which this ion are linked must remain subjects of speculation. Normal muscle contains large quantities of phosphate, chiefly in hexose and triose esters, adenine nucleotides, creatine phos-

phate, and phospholipids. Most of this phosphate is not histochemically detectable in fixed material because, being of relatively small molecular size, these compounds are diffusible and relatively soluble. At any rate, normal muscle in the same section is practically uncolored by the technic which reveals well marked phosphate-positive necrotic segments. The concentration of phosphate on or in the basophilic striae derived from the altered myofibrils in the earlier stages of segmental degeneration of muscle fibers is noteworthy. Such striae are the site of simultaneous localization of both bound ionizable phosphate and cations.

It would seem that extensive mineralization follows consequent to the earlier accumulation of ionizable phosphate groups in the altered myofibrils, which thereby assume an acidic function and accept basic dyes. Muscle degradation involves profound changes in distribution of minerals. That the mineral deposits should contain so much iron (rather than calcium) may perhaps be accounted for by the presence of considerable iron in striated muscle in the form of easily freed labile iron[24] as well as in myohemoglobin. The concentration of labile iron in muscle is three or four times greater than in the blood.[27] In fact the ash of micro-incinerated striated muscle fibers does exhibit the yellow color indicative of iron,[28] as might be expected from the quantities discovered in gross chemical analyses. It may be that iron derived from myohemoglobin participates in this dystrophic siderosis.

It is remarkable that extensive dystrophic mineralization, as observed in the muscles of many specimens, should occur so soon after injury. Schujeninoff[29] long ago called attention to the early calcification of muscle wounds. In both experimental animals and postoperative human material he observed, using the gypsum method, deposition of calcium salts in quantity as early as 18 hours after injury.

Regeneration

Tinctorial Properties. The general tinctorial and structural characteristics of the differentiating sarcoblasts have been described. In the young proliferating sarcoblasts the peculiar form of the nucleoli and the marked cytoplasmic basophilia are especially noteworthy. When stained with relatively dilute solutions of methylene blue, the cytoplasm appears a rich blue or violet-blue. This basophilia gradually diminishes as differentiation proceeds and as myohemoglobin is added, and as the nuclear form reverts to the adult type (Fig. 3).

Nucleic Acids. The Feulgen reaction is not given by any extranuclear component of the regenerating muscle. Pre-treatment of sections with solutions of crystalline ribonuclease completely removes the af-

finity of the cytoplasm of regenerating muscle for basic dyes, and significantly diminishes the staining capacity of the nucleoli with these dyes. The presumptive conclusion is that ribonucleic acid or ribonucleoproteins are responsible for the cytoplasmic basophilia and are present in the nucleoli.

Phosphatase. Using a single substrate (glycerophosphate) at pH 9.0 only very slight phosphatase activity was detected in the cytoplasm of sarcoblasts, the nuclei showing stronger coloration. At pH 4.5, moderately strong phosphatase activity was found to occur diffusely throughout the cytoplasm, which was a uniform brown, and also rather strongly in the nuclei of young sarcoblasts. The precipitate was found only in the nuclei of matured fibers. Control slides incubated without substrate failed to develop any coloration.

Discussion of Regeneration

From the structural configuration of the nucleus, and the large amounts of ribonucleic acid in the cytoplasm, the young sarcoblast appears to be a unit organized for intensive cytoplasmic protein synthesis. It is supposed to be characteristic of such cells to show conspicuously increased nucleolar masses containing ribose nucleotides, and high concentrations of ribonucleotides in the cytoplasm.[30,31] The extraordinary nucleolar form and enlargement in the young sarcoblast which has been described ("rhombosome") coincide with the period of maximal cytoplasmic basophilia. During this time the sarcoblast must simultaneously meet the protein requirements of both extremely rapid growth and differentiation, with myofibril and sarcolemma formation. From the data which have been presented on the rate of growth,[1] which is great, and the speedy appearance of myofibrils, it is evident that regenerating muscle has distinct embryonal characteristics in these respects, as reflected in the high concentration of cytoplasmic ribonucleic acid and in the nucleolar size. Other regenerating tissues are known to have similar characteristics.[31]

Cytoplasmic basophilia persists for some time after the recession of nucleolar size in the maturing fiber. It begins to disappear as the first eosinophilic material, probably including myohemoglobin, is formed. Such fibers already have cross-striated myofibrils, Z disks, and sarcolemma. The character of the cytoplasmic basophilia with methylene blue is worthy of mention, for in the young sarcoblasts it is sometimes found to have a slightly violet tint, *i.e.*, there is metachromatic staining. This quality of staining as assumed by ribonucleic acids is well known.[32-34]

The demonstration of acid phosphatase in the ribonucleotide-rich

slips of regenerating muscle is of particular interest. The association of increased phosphatase activity with accumulations of cytoplasmic ribonucleic acid has been observed in a number of situations (neuronal axon regeneration, sites of collagen formation, in osteoblasts, in silk glands), which have in common the synthesis of fibrous proteins (Bradfield[35]). Cytoplasmic phosphatase is generally absent in cells concerned with the secretion of globular proteins, although high concentrations of ribonucleic acid are present. In this connection the sarcoblast is intensively engaged in the new formation of the fibrous protein myosin, and it is just preceding and during the first appearance of the myofibrils that acid phosphatase activity and high concentrations of ribonucleotide are simultaneously demonstrable in the sarcoblastic cytoplasm.

Fat

Necrosis of the maturing fetal fat pads is one of the most conspicuous lesions of suckling mice infected with certain strains of Coxsackie virus (*viz.*, Conn.-5, Ohio-1, and Nancy). The acute destructive lesion has the peculiarity of being confined chiefly to a peripheral band surrounding each lobule of the affected fat. In this zone there is smudgy necrosis of fat cells which appear opaque, granular, and fused. Mineralization occurs early. Subsequent events include the appearance of giant cells and new fibrous tissue, as described for other destructive lesions in adipose tissue.[36,37]

Tinctorial and Optical Properties

The freshly necrotic peripheral bands were composed of discrete or fused cells containing numerous refractile, angular granules varying in size from 2 to 4 μ. These surrounded single or multiple, small, central vacuoles which enclosed acetone-extractable sudanophilic globules; in unstained frozen sections the inner aspect of these vacuoles appeared blurred due to the deposition of very fine crystals which lay perpendicularly to the surface. The larger refractile granules were markedly hematoxylinophilic, and with the basic dyes most of the finer granules were stained against a more diffuse tinting of the background cytoplasm. These affinities persisted after acetone extraction. Under the polarizing microscope there was an indiscriminate and sparse scattering of fine birefringent crystals throughout the lobule, without any consistent localization; these disappeared when the slide was warmed to 37° C., and after acetone extraction.

By the third day of survival most of the granules in the peripheral parts appeared as large, sharp, crystalline blocks, averaging 4 to 6 μ,

which were agglomerated into clumps or masses persisting in clusters about small fat-containing globules. They remained hematoxylinophilic, but basophilia was now seen only in the smaller granules, and faintly in the large, anuclear, circumscribed, non-granular masses which appeared in abundance in the affected zones at this time. These bodies were streaked with sudanophilic material. Sections of lesions at this stage of development stained with Nile blue sulfate showed the relatively unaffected interiors of the lobules in the rose color presumably characteristic of unsaturated glycerides, while in the involved peripheral bands there were many bodies ranging in tint from mauve or lilac to blue, as well as scattered rose-colored globules. After acetone extraction only blue-stained bodies and granules remained.

From the fifth day of survival, the lesion was dominated in most instances by obvious mineralization in the form of chalky masses. Fibrosis also became evident. In those instances in which mineralization was less marked, large, faintly basophilic, anuclear bodies were prominent in the granulomatous zones.

Intravital Trypan Blue. The necrotic peripheral bands of the diseased fat lobules were precisely designated by their blue coloration with trypan blue, while the viable remainder of the lobules was unstained (Fig. 1). The altered fat cells appeared rounded and composed of dark blue granules and masses disposed as bridges enclosing two or three separate vacuoles. In about one half of the cells, dark stained distorted nuclei were still evident.

Feulgen Reaction. In attempting to identify some of the basophilic components in the necrotic areas, the presence of desoxyribonucleic acid was sought. The acute lesions of the recently affected fat lobules were selectively, diffusely, and prominently colored by the Feulgen reaction, the control fat from normal animals remaining unstained except for the nuclei. The Feulgen-positive material in these areas usually was found as a diffuse, somewhat faint blush or smudge in the degenerated elements or fused cell masses, as sharply stained, small, irregular (2 to 3 μ) granules, and in the shrunken and fragmenting nuclei (Fig. 7). In the older lesion (3 to 4 days of survival after first signs) there was a marked decline of extranuclear Feulgen stainable material. It was now confined almost entirely to the large, fused, anuclear masses, where it was seen as a faint, diffuse, uneven background and scattered, small, intensely stained granules. These anuclear masses persisted to the seventh day after the onset of sickness, beset with giant cells and mononuclear cells, still faintly but definitely Feulgen positive, and containing Feulgen positive granules (Fig. 8).

Minerals

Early mineralization is one of the most obvious features of the steatitis. It may be grossly apparent even on the first or second day of signs, and by the fifth day the affected interscapular fat pads are usually crumbling chalky masses. The nature and localization of some of the inorganic components of these deposits were studied.

Phosphate. As might be anticipated from the distribution of desoxyribonucleic acid, a positive reaction for phosphates was obtained in the acute lesion corresponding to the areas of necrosis. Ionizable phosphate was found in quantity in both granular and diffuse form in the degenerating elements as well as diffusely in some of the interstitial tissues, suggesting a freely diffusible phosphate-containing substance. The von Kossa and Laidlaw silver stains usually were strongly positive in the necrotic zones, presumably owing to the presence of phosphate. Some diffused inorganic phosphate was still apparent in fat lesions at least 3 days old, together with many phosphate-positive granules. In still older lesions (5 to 7 days), however, the phosphate appeared to be confined to the granules and masses which were also shown to be calcified.

Carbonate. Carbonate, which was not found in the early acute lesions, was first detected in lesions at least 3 days old, and persisted thereafter in older specimens. When present it was found in considerable amount, always associated with large deposits of calcium.

Calcium. Small amounts of calcium were discovered in lesions in the acute stage. In exceptional cases, mineralization may be marked even on the first day, but ordinarily large deposits of calcium salts were found on the third day, as granular masses, which became augmented in older specimens. Calcium salts were thus the principal mineral substance.

Iron. Ferrous and ferric iron appeared to have the same distribution in the affected lobules. Until calcification becomes marked, iron is found in very small or trace amounts in the form of small granules in the dead or dying fat cells at the extreme periphery of the lobules, and only in some sectors. In the older specimens with obvious lime deposits there was more iron, chiefly as granules in the peripheral rim, in the same regions as the calcium salts.

Discussion of the Lesions in Adipose Tissue

Considerable interest has always attached to the course of events in the evolution of destructive inflammatory lesions of adipose tissue, because of the long persistence of exudate and reparative activity. In

this respect the reaction to injury in fat tissue differs from that of other tissues. The reasons for this are not entirely clear, but evidently are related to chemical alterations of the neutral fat. Information concerning the histologic features of these lesions has been derived from injection of foreign lipid substances or observation of cases of endogenous fat necrosis. The lipotrophic strains of Coxsackie virus provide a new and ready means for the experimental study of the course and development of non-traumatic endogenous steatitis.

Among the anatomical problems which this lesion presents is the limitation of necrosis to the periphery of the lobule in the recent or acute specimens. The only reason that suggests itself is that this zone is farthest removed from the main nutrient vessels, which are mostly central. In the most acute lesion observed, the cytoplasm (relatively abundant in the embryonal fat) of the necrotic fat cells contains much diffuse and granular basophilic material, evidently desoxyribonucleic acid derived from nuclear disintegration, and probably its salts. The abundant ionizable phosphate must be derived at least in part from this nucleic acid. The liberation of desoxyribonucleic acid from nuclei in areas of cell death has been described as occurring in a variety of circumstances.[38] Unequivocal evidence for the presence of free fatty acid was not obtained, but the minute parallel crystals seen about the vacuoles in unstained frozen sections and the basophilic granular and amorphous material suggest its formation. The scattered birefringent crystals can be designated only as crystalline lipid, not necessarily fatty acid. Preparations stained with Nile blue sulfate indicate only a profound change in the fat of the affected part, without permitting any further chemical deductions. The somewhat basophilic Feulgen-positive anuclear masses which have been described, and which are usually found beset with giant cells, may represent in part an amorphous semisolid material formed in the presence of unsaturated fatty acids or their methyl esters.[39,40] According to Hass,[39] such material is the only effective stimulus to giant cell formation; it apparently resists solution by alcohol and xylol. The acidic substances evidently bind available cations from the tissue fluids, leading to the very early deposition of minute crystals, chiefly calcium salts, in the perivacuolar cytoplasm. It would seem clear that in these lesions cell death precedes and is the cause of subsequent chemical changes, including lipolysis. In the further course of the lesion, phosphate increases as Feulgen detectable nucleic acid declines, and the crystalline mineral deposits increase. Although microscopic mineralization appears always to occur following alterations of the endogenous fat tissue, massive mineralization does not necessarily take place. Calcification is apparently not an essential

feature of traumatic lipogranuloma.[37] The occurrence of associated iron and calcium deposits has been commented upon by Bunting.[8]

The pathogenesis of the granulomatous lesions following fat necrosis presents a number of unsolved problems.[37] Depending largely upon a specific histochemical interpretation of the Nile blue sulfate stain, Lecène and Moulonguet[41] proposed that intracellular saponification is the fundamental lesion, even preceding cell death, and evoking a chronic foreign body inflammatory reaction. Their reliance upon the Nile blue sulfate stain for chemical information has not been justified by subsequent knowledge of the non-specificity of this procedure for the detection of fatty acids and soaps. The supposition that fatty acids and soaps are always responsible for the lipogranulomatous process is weakened by the fact that injected unsaponified oils produce a similar chronic reaction with fibrosis.

In virus-produced endogenous fat lesions, cell death, which is the primary event, leads to a local deposition of substances (some of which rapidly bind cations) which precedes and seemingly provokes the granulomatous foreign body reaction. Some of these deposits are most probably lime soaps, although the acute suppurative response said to be associated with the highly irritating action of soaps and of the products of hydrolysis of fat[37] was not evident. Most of the calcium is probably laid down as phosphate, particularly in the later stages.

Summary

Some of the characteristics of the degenerating and regenerating striated muscle, and of the lesions of the fat bodies occurring in the course of Coxsackie virus disease in infant mice were studied, applying histochemical technics. Animals infected with Conn.-5, Ohio-1, Texas-1, and Nancy strains of virus, all antigenically distinct, were included in the study.

Segments of muscle in the early stages of degeneration were found to show apparently accentuated, positive birefringence, uninterrupted by cross striations. Anisotropy was generally lost as hyalinization advanced.

Necrotic muscle segments often showed basophilia, corresponding to the sites of deposition of ionizable phosphates. Early mineral deposits, chiefly iron, were found along the altered myofibrils which form the longitudinal striae of the early stages of segmental muscle degeneration. Heavy mineralization often was found as early as the first signs of overt disease.

The plasmodial sarcoblasts of the regenerating muscle were found to have characteristically large nucleoli and intensely basophilic cyto-

plasm, which was the site of concentrations of ribonucleic acid and acid phosphatase, prior to and during the first appearance of myofibrils and myohemoglobin.

The necrosis in viral steatitis is confined chiefly to a peripheral band surrounding each lobule. The necrotic regions were found to contain diffuse Feulgen-positive material derived from nuclear disintegration. Giant cells and new fibrous tissue develop as in other destructive lesions of adipose tissue.

Very early mineralization, sometimes massive, was the rule in these sites, which were found to contain diffuse and granular phosphate, calcium, and in the late stages, carbonate. Iron was present in trace amounts. Suggestive evidence for the presence of free fatty acids was obtained.

ADDENDUM

Since this paper was submitted, a study by Kausche, Landschütz, and Sauthoff[42] on the histochemical demonstration of alkaline phosphatase in the muscle lesions of Coxsackie virus disease has come to our attention. These authors reported alkaline phosphatase activity in the degenerating segments of muscle of mice infected with the Coxsackie virus, and concluded that Coxsackie virus activates phosphomonoesterase in muscle. We have repeated our observations on a series of new preparations of affected muscle dissected from bone from Texas-1 and Conn.-5 infected mice. In every case the results were the same as those described in the text. Since our controls show the same localization of metallic precipitate in the muscle, it is impossible to assess the actual phosphatase activity of the diseased segments.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

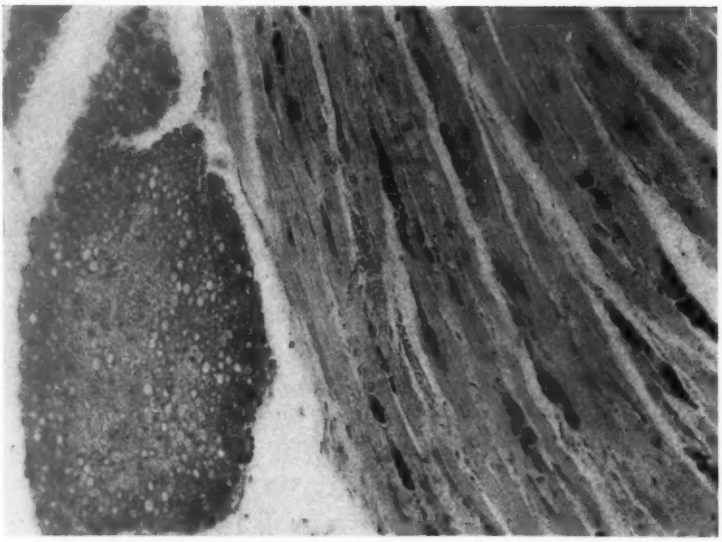
PLATE 82

FIG. 1. Muscle and fat lobule, stained intravitally with trypan blue to demonstrate the distribution of affected elements in the early lesions. The necrotic parts are seen as darkly stained. The widespread scattering of segmental necrosis in the muscle is evident; many hyalinized segments appear comminuted. The characteristic peripheral distribution of necrotic cells is apparent in the fat lobule. Conn.-5 strain, first day of signs. × 200.

FIG. 2. Heavy metallic deposits on the necrotic fiber segments in a section of an affected muscle stained according to the Gomori method for alkaline phosphatase, but without the substrate. Sections exposed to substrate present identical appearances. The dark precipitate presumably indicates the presence of "preformed" ionizable phosphate in the necrotic segments. Conn.-5 strain, first day of signs. Gomori method. × 600.

FIG. 3. Sarcoblastic slips, exhibiting the characteristic nuclear form with large nucleoli, and basophilic cytoplasm. Ohio-1 strain, fourth day after onset of signs. Dilute methylene blue stain. × 800.

1



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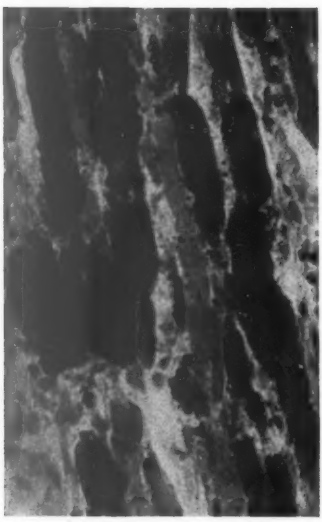

3

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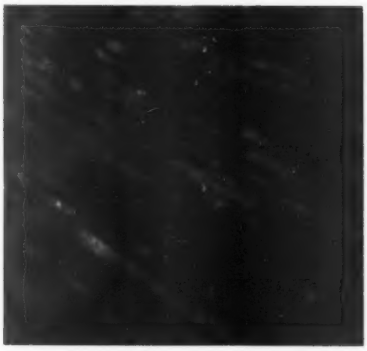
PLATE 83

FIG. 4. Birefringence of degenerated muscle fiber segments. Texas-1 strain, first day of signs. Unstained frozen section photographed through microscope with crossed prisms. × 100.

FIG. 5. Concentration of ionizable phosphate in the degenerated muscle segments, within which the reaction for phosphate is more pronounced in the longitudinal striae. Texas-1 strain, first day of signs. Molybdenum blue method. × 800.

FIG. 6. Degenerated muscle segment stained to show distribution of minerals (iron and calcium) within the fiber segment. The longitudinal striae derived from the myofibrils appear selectively mineralized. Easton strain, second day of signs. Gallamine blue stain. × 800.

4



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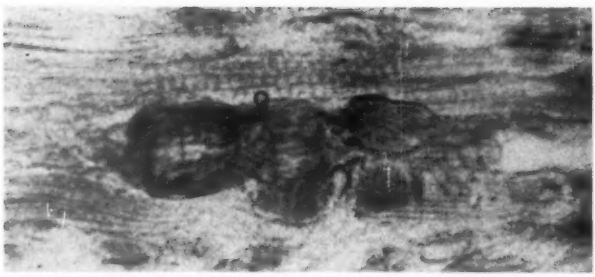

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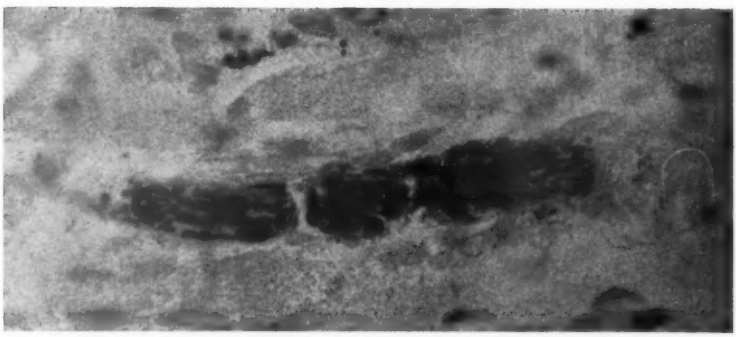


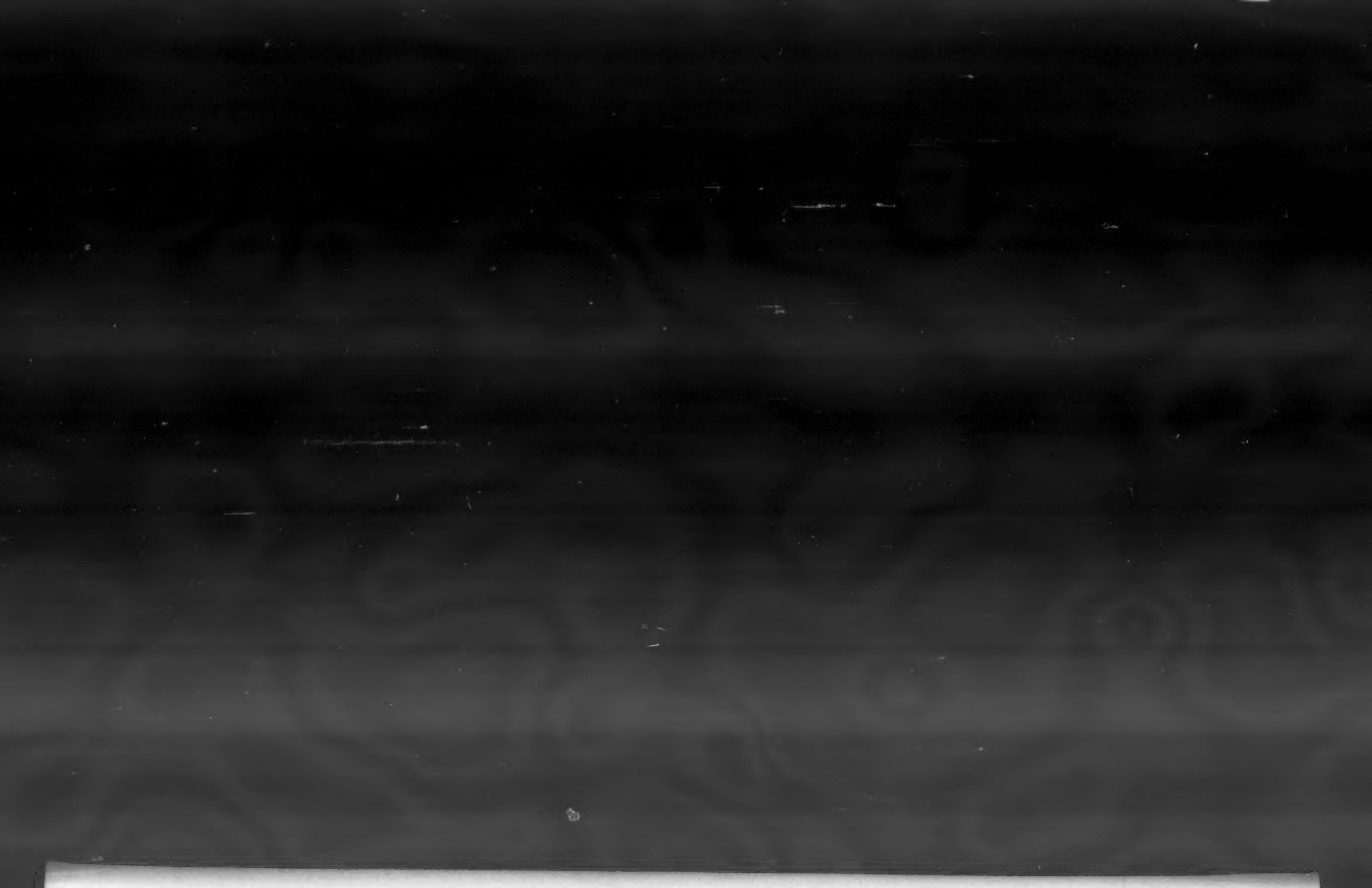
PLATE 84

FIG. 7. Periphery of necrotic fat lobule showing the diffusion of desoxyribonucleic acid in the peripheral zone composed of partly fused necrotic fat cells. Ohio-1 strain, first day of signs. Feulgen technic. × 515.

FIG. 8. Fused anuclear masses containing desoxyribonucleic acid in a more advanced lesion of a fat lobule which shows a granulomatous reaction. The Feulgen-positive masses are surrounded by some giant cells. Ohio-1 strain, fourth day after onset of signs. Feulgen technic. × 515.

FIG. 9. Granular mineral deposits, chiefly calcium and traces of iron, within the necrotic fat cells at the periphery of a lobule. Most of the spherical clear zones represent lipid globules. Ohio-1 strain, fifth day after onset of disease. Gallamine blue stain. × 335.

FIG. 10. Refractile mineral masses at the periphery of an affected fat lobule, showing more massive mineralization of necrotic elements and some stages in its development. The darkly stained material is lipid. Ohio-1 strain, seventh day after onset of disease. Sudan IV stain. × 335.



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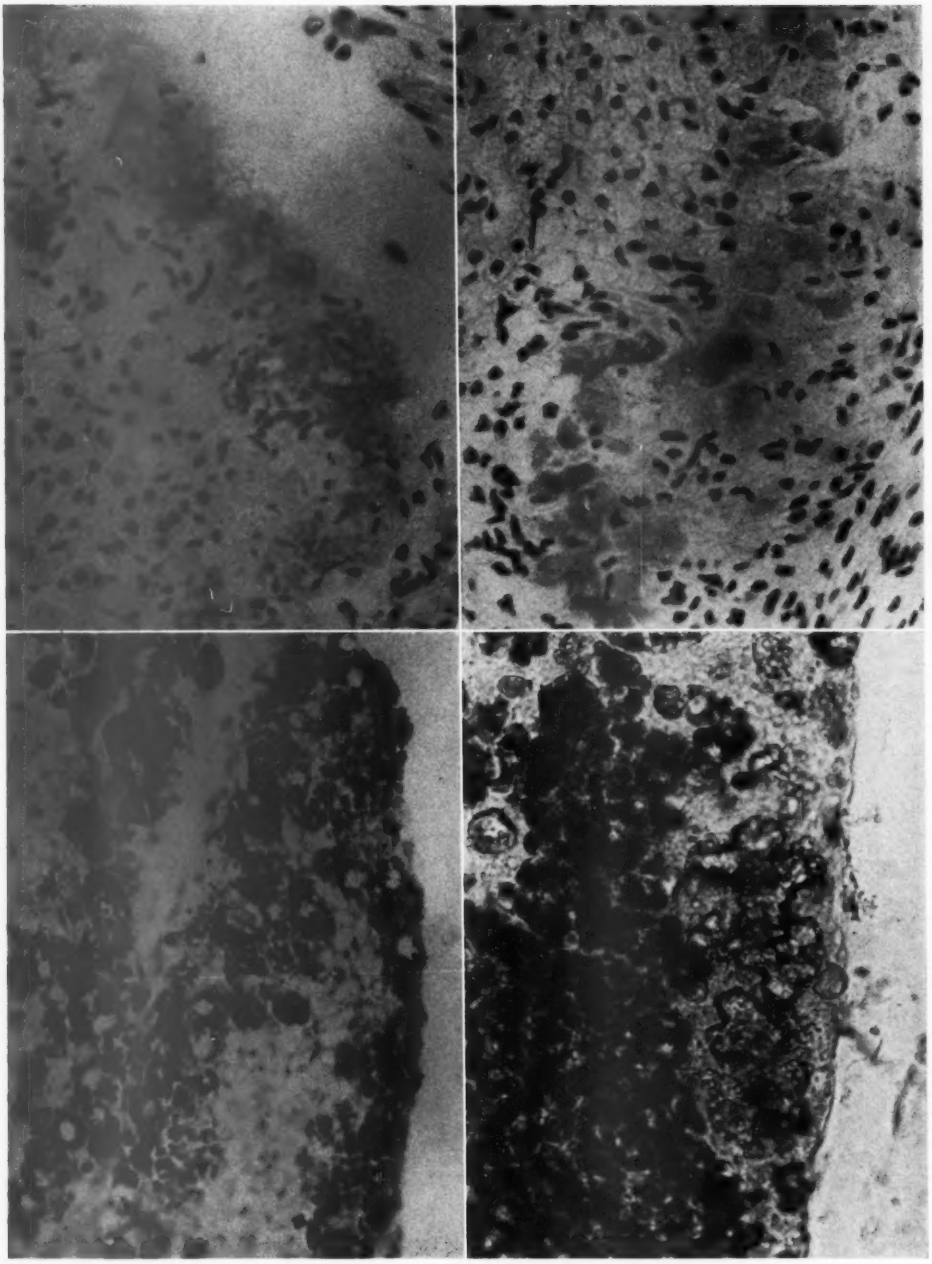

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Godman, Bunting, and Melnick Coxsackie Virus: II. Histochemical Observations

NOCARDIOSIS

Nocardial Osteomyelitis and Septicemia*

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In 1888, Nocard[1] described an acid-fast actinomyces causing a disease in cattle known as farcin du boeuf. Three years later, Eppinger[2] described the first human case of acid-fast actinomycosis in a man, 52 years old, whose death was due to a brain abscess. Because the organism isolated from the brain abscess and meningeal pus produced false branching and star-like colonies in agar, he called it *Cladothrix asteroides*. Trevisan[3] was the first one to call the organism *Nocardia asteroides*.

Subsequently there have been reports of a number of human cases of acid-fast actinomycosis. In 1921, Henrici and Gardner,[4] reviewing the world's literature, found 25 cases of infection by *N. asteroides* in man. Sixteen were of the pulmonary type with lesions ranging from bronchopneumonia to abscess formation; 5 had cerebral lesions with or without accompanying meningitis; 2 were cases of peritonitis following abdominal operations; 1 was a case of spinal cord abscess; and 1 was a case of cellulitis of the foot (Madura foot). Of these 25 cases, only one recovery was listed, that of Madura foot in a Filipino housewife, 30 years old, following amputation of the affected extremity. In 17 cases, cultures were positive for the organism but in 7 cases the typical lesions were present but no cultures were obtained. To these 25 cases, Henrici and Gardner in the same paper added another case of nocardiosis in a woman, 31 years of age, who was admitted with pulmonary symptoms and who apparently improved when given an autogenous vaccine. Henrici and Gardner did not include 2 other cases of nocardiosis. One was reported by Steele and Lee[5] in 1913 in a schoolboy, 19 years old, who developed right lobar pneumonia with empyema. The organism was isolated from the sputum and the patient made a spontaneous recovery after thoracotomy and drainage. The other case was that of Davis,[6] in 1914, whose patient showed consolidation of the lung and who apparently improved with potassium iodide.

In 1937, Goldsworthy[7] added a fatal case of pulmonary infection by Nocardia. The following year, Kessel and Goolden[8] described another fatal case of pulmonary infection with meningitis. In 1944, Benbow, Smith, and Grimson[9] for the first time reported 2 cases of pulmonary infection treated with sulfonamide, surgical drainage, roentgen rays,

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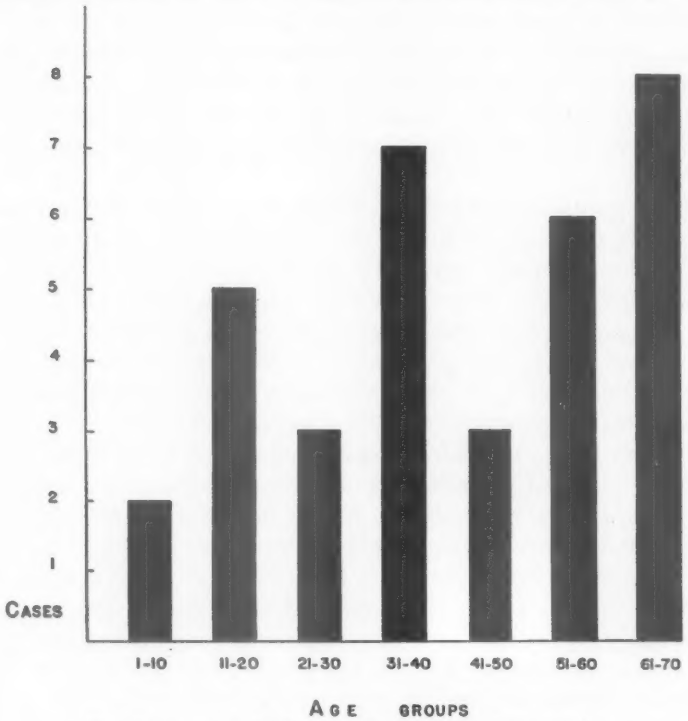
iodides, vitamins, and bed rest with subsequent recovery. A year later, Binford and Lane[10] isolated the organism at necropsy from chronic suppurative pneumonitis, massive cerebral abscess, and an ischiorectal abscess. In 1946, Kirby and McNaught[11] reported 2 cases, one of pulmonary infection and one of cerebral abscess. The patients were



Text-figure 1. Age distribution of human cases of nocardiosis.

treated with sulfadiazine but died. In the same year, Shaw, Holt, and Ray[12] reported a case of pulmonary infection successfully treated with penicillin, thymol, potassium iodide, sulfadiazine, and surgical drainage of an empyema cavity. Calero,[13] in the same year, recorded the case of a 12-year-old girl with lung infection who was considered cured after sulfonamide and penicillin therapy. In 1948, Glover, Herrell, Heilman, and Pfuetze[14] described a pulmonary case with empyema successfully treated with sulfadiazine orally and intrapleurally after penicillin and streptomycin had failed. Hager, Migliaccio, and Young[15] reported 2 cases in 1949. One case, of the pulmonary type, was treated and cured with penicillin, streptomycin, and surgical drainage. The

TABLE I

Summary of the Reported Cases of Nocardiosis in Man

Case no.	Reported by	Sex	Age	Organs Involved	Culture	Duration	Outcome	Therapy	Origin
1	Eppinger,[2] 1890	M	52	Brain, lungs, pleura, peribronchial lymph node	Positive from brain	12 days	Died		Graz, Germany
2	Ferré and Faguet,[18] 1895	M		Brain	Positive from brain (post mortem)		Died		France
3	Sabrazès and Rivière,[19] 1895	M		Brain, kidneys, lungs	No culture		Died		France
4	Sabrazès and Rivière,[19] 1895	M	12	Lung, subcutaneous tissue	Positive from sputum and subcutaneous abscess		Died		France
5	Berestneff,[20] 1897			Brain, lung	No culture		Died		Russia
6	Ljubimoff,[21] 1897			Lung	No culture		Died		Russia
7	Scheele and Petruschky,[22] 1897	F	56	Lung, subcutaneous tissue	Positive from sputum and subcutaneous abscess	7 months	Died		Danzig, Poland
8	Buchholz,[23] 1897	M	38	Lung	No culture	23 days	Died		Berlin, Germany
9	Flexner,[24] 1898	M	70	Lung, peritoneum, omentum	No culture		Died		Baltimore, Md., U.S.A.
10	Aoyama and Miyamoto,[25] 1900	M	35	Brain, lungs	Positive from sputum and lung abscess	31 days	Died		Japan
11	Musser,[26] 1901	M	37	Lung	No culture	3 months	Died		Philadelphia, Pa., U.S.A.
12	MacCallum,[27] 1902	M	3	Peritoneum, liver capsule (post-gastrostomy)	Positive from peritoneal fluid	23 days	Died		Baltimore, Md., U.S.A.

TABLE I (Continued)

Case no.	Reported by	Sex	Age	Organs involved	Culture	Duration	Outcome	Therapy	Origin
13	Birt and Leishman,[28] 1902	M	26	Lung	Positive from sputum and empyema fluid	6 months	Died	Aspiration of empyema cavity	South Africa
14	Horst,[29] 1903	M	33	Brain, lung	Positive from brain and lung	18 days	Died		Germany
15	Schabad,[30] 1904	M	62	Lungs, pericardium	Positive from sputum and pleural fluid	3 years	Died		St. Petersburg, Russia
16	Stokes,[31] 1904	M	28 days	Lung	Positive from lesion (post mortem)		Died		Baltimore, Md., U.S.A.
17	M'Donald,[32] 1904	F	65	Lung, kidney, brain, heart	Positive from lesion (post mortem)	6 months	Died		Edinburgh, Scotland
18	Butterfield,[33] 1905	M	19	Lung	No culture	3 years	Died	Codeine, arsenic	Washington, D.C., U.S.A.
19	Musgrave and Clegg,[34] 1907	F	30	Right foot	Positive from sinus discharge from foot	3 years	Recovered	Amputation	The Philippines
20	Loehlein,[35] 1907	M	58	Brain, lung, liver, spleen	Positive from brain (post mortem)		Died		Leipzig, Germany
21	Bernstein,[36] 1909	M	67	Lung, peritoneum	Positive from lung	5 weeks	Died	Paracentesis abdominis	London, England
22	Foulerton,[37] 1910	M	55	Peritoneum (post-appendectomy)	No culture	24 days	Died		England
23	Foulerton,[37] 1910	M	50	Lung	No culture	1 year	Not reported		England
24	Steele and Lee,[5] 1913	M	19	Lung	Positive from sputum	6 months	Recovered	Thoracotomy and drainage, instillation of bismuth paste into empyema cavity	Boston, Mass., U.S.A.
25	Davis,[6] 1914	M	64	Lungs	Positive from sputum	4 weeks	Improved	Potassium iodide	Chicago, Ill., U.S.A.

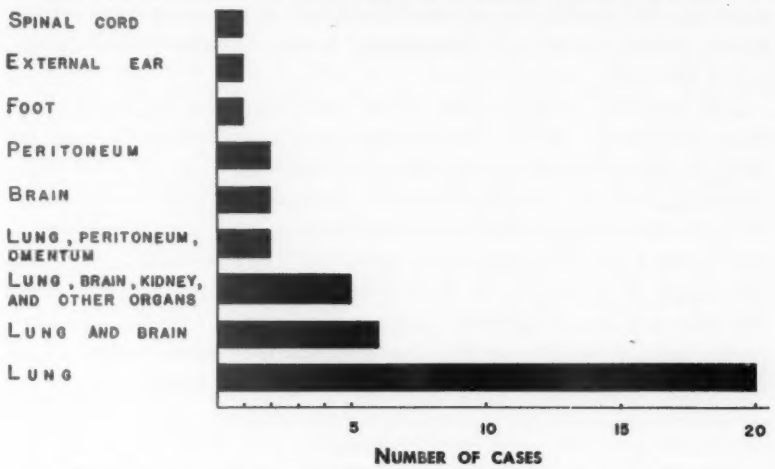
								bismuth paste into empyema cavity	
25	Davis,[6] 1914	M	64	Lungs	Positive from sputum	4 weeks	Improved	Potassium iodide	Chicago, Ill., U.S.A.
26	De Korté,[16] 1917	M	50	External ear	Positive from aural sinus discharge	3 years	Recovered	Potassium iodide, autogenous vaccine	South Africa
27	Henrici and Gardner,[4] 1921	F	31	Lung	Positive from sputum	4 years	Living with symptoms	Autogenous vaccine	Rochester, Minn., U.S.A.
28	Ribytkof and Maloletkof[38]			Spinal cord	No culture		Died		
29	Goldsworthy,[7] 1937	M	69	Lung, subcutaneous tissue, kidney, adrenal gland	Positive from sputum	1 month	Died		Australia
30	Kessel and Goolden,[8] 1938			Lung, meninges			Died		Los Angeles, Calif., U.S.A.
31	Benbow, Smith, and Grimson,[9] 1944	F	36	Lung, subcutaneous tissue (neck, shoulder, hips)	Positive from sputum and abscess from back	13½ months	Recovered	Sulfanilamide, potassium iodide, surgical incision and drainage, roentgen ray therapy, blood transfusion, supportive therapy	North Carolina, U.S.A.
32	Benbow, Smith, and Grimson,[9] 1944	M	52	Lung, chest wall, perirectal area	Positive from sputum	13 months	Recovered	Sulfathiazole and sulfadiazine, potassium iodide, roentgen ray therapy, surgical incision and drainage	North Carolina, U.S.A.
33	Binford and Lane,[10] 1945	M	51	Lung, brain, ischiorectal region	No culture	40 days	Died	Barbiturate, aspirin, codeine	New Orleans, La., U.S.A.
34	Kirby and McNaught,[11] 1946	M	63	Lung, subcutaneous tissue, thyroid gland, spleen, kidneys, ileum	Positive from sputum	4 months	Died	Sulfadiazine, roentgen ray therapy	Central California, U.S.A.

TABLE I (Continued)

Case no.	Reported by	Sex	Age	Organs involved	Culture	Duration	Outcome	Therapy	Origin
35	Kirby and McNaught,[11] 1946	F	49	Cerebellum	Positive culture from brain abscess (post mortem)	1 month	Died		Stockton, Calif., U.S.A.
36	Shaw, Holt, and Ray,[12] 1946	F	34	Lung, buttocks	Positive from pleural fluid	16 months	Recovered	Penicillin, thoracotomy and drainage, potassium iodide, thymol, sulfadiazine, blood transfusions	Virginia, U.S.A.
37	Calero,[13] 1946	F	12	Lung	Positive from pleural fluid and sputum	7 months	Recovered	Sulfonamide, penicillin, supportive therapy	Panama
38	Glover, Herrell, Heilman, and Pfuetze,[14] 1948	M	26	Lung	Positive from empyema fluid	4 years	Recovered	Sulfadiazine (orally and intrapleurally). Penicillin, and streptomycin tried without success	Rochester, Minn., U.S.A.
39	Hager, Migliaccio, and Young,[15] 1949	F	16	Lung	Positive from empyema fluid	2½ months	Recovered	Penicillin, streptomycin, thoracotomy and drainage, sulfadiazine	Rhode Island, U.S.A.
40	Hager, Migliaccio, and Young,[15] 1949	F	64	Lung	Positive from sputum	3 years	Outcome unreported	Organism sensitive to penicillin, aureomycin, and streptomycin	Rhode Island, U.S.A.

second was also a pulmonary case in which the organism was also found to be sensitive to penicillin, but the outcome was not stated.

Up to 1949, 40 cases of nocardiosis had been reported. These include a case of otomycosis[16] caused by an acid-fast bacillus called *Nocardia cylindracea,* which improved after treatment with potassium iodide and autogenous vaccine. This case occurred in South Africa some time in



Text-figure 2. Distribution of cases of nocardiosis according to organs involved.

1917. In 1949, a case of granulomatous nocardiosis with intracellular parasitism supposed to be caused by *Nocardia intracellularis,* a hitherto unknown species, was reported by Cuttino and McCabe.[17]

In reviewing the 40 reported cases of nocardiosis in man (Table I), several significant features become evident. In the first place, the distribution appears to be world-wide, with cases reported in the United States, Panama, East and West Europe, England, the Philippines, South Africa, Australia, and Japan. This is not surprising since acid-fast actinomyces pathogenic to experimental animals have been isolated from soil.[39] Racial distribution cannot be stated since many of the reported cases fail to mention the race of the patient.

As to age, there is a wide range of from 28 days to 70 years. Of the 34 reported cases stating the patients' ages (Text-fig. 1), the average age is between 35 and 40 years. Of the 36 cases giving the patients' sex, 26 were males (72.2 per cent) and 10 were females (27.8 per cent). This may be related to the fact that the organism lives and thrives in soil,[39] and men rather than women work with soil.

The disease is highly fatal. Of the 40 cases reported, only 9 patients were listed as definitely recovered (22.5 per cent), 27 were listed as

having died (67.5 per cent), the outcome of 2 was not reported, and 2 were improved with the therapy given but not cured. Of the 9 recoveries, 6 occurred after the year 1944 and were attributed to the use of sulfonamides, antibiotics, and surgical incision and drainage. Of the 3 recoveries reported before this era, one patient was cured by amputation of the affected foot,[34] the second recovered after thoracotomy, drainage, and instillation of bismuth paste into the empyema cavity,[5] and in the third recovery was attributed to potassium iodide and autogenous vaccine.[16]

The organism shows a very strong predilection for the respiratory tract. Of the 40 cases in the medical literature (Text-fig. 2), 24, or 60.0 per cent, showed primary lung involvement, 9, or 32.5 per cent, showed lesions in both the lungs and the brain, 2 showed localization in the brain, 2 revealed peritoneal involvement, 1 was a case of Madura foot, 1 was a case of spinal cord abscess, and 1 of external ear infection. Five cases, or 12.5 per cent, showed involvement of viscera other than the lung and brain, suggestive of a hematogenous spread. The preponderance of pulmonary infection suggests that the organism probably enters the body more frequently through the airways.

Report of Case

E. H. (Pennsylvania Hospital no. 93904) was a married colored female, 43 years old, who had been born in Maryland. She was acutely ill when admitted on March 3, 1951, with the chief complaint of pain in the right thigh. This pain started 6 weeks prior to admission and was accompanied by some loss of weight. Oral medication, the exact nature of which was unknown, had afforded only transient relief. The pain in the right thigh continued intermittently and during the 2 weeks prior to admission it became so severe that she had to use a cane, and was completely bedridden 2 days prior to admission. She had complained of soreness of the mouth for the few days before admission. She had fever, night sweats, and chilly sensations, but there was no history of cough or hemoptysis. Temperature on admission was 103° F.; pulse, 120 per minute; respirations, labored and rapid with a rate of 30 per minute; blood pressure, 130/70 mm. of Hg. Physical examination revealed marked wasting. There were adherent white plaques over the soft and hard palate, pharynx, and tonsillar walls. There was a grade III blowing systolic murmur at the apex transmitted over the entire precordium. The lungs were clear. A questionable right costovertebral angle tenderness was elicited. There was acute tenderness with swelling and induration over an anterolateral area measuring 7.5 by 10.0 cm. at the junction of the upper and middle thirds of the right thigh, but there was no impairment of hip or knee motion. In both axillae there were a few non-tender, enlarged lymph nodes. The impression was osteomyelitis of the right femur and oral and pharyngeal moniliasis since branching filaments were obtained by swabbing the mouth and pharynx. Because of the cardiac findings, bacterial endocarditis was considered also.

Laboratory Findings. Routine blood count revealed slight anemia (red blood cells from 3.0 to 3.5 millions per cc. with hemoglobin of 10 to 11.0 gm.); a moderate leukocytosis ranging from 11,000 to 15,000 with polymorphonuclear cells of 68 to 79 per cent. The sedimentation rate was rapid (40.0 mm. per hour). Eagle and Wassermann tests of the blood were negative. Blood culture done three times revealed

negative findings after 7 to 8 days. Urinalysis showed slight albuminuria toward the latter part of the patient's illness, with few to 20 red blood cells and abundant white cells in her urine. Blood agglutination tests using typhoid "O," typhoid "H_2," paratyphoid A, paratyphoid B, Proteus OX_{19}, and *Brucella abortus* were negative. Chemical examination of the blood showed increasing blood urea nitrogen and creatinine reaching a maximum of 53 and 1.8 mg. per cent, respectively, on April 4, 1951.

Course in the Ward. A smear of the white plaques in the patient's mouth and throat done immediately after admission showed numerous branching, non-budding filaments. A roentgenogram of the right thigh taken about the same time was diagnosed as osteomylitis of the right femur of several weeks' duration. Roentgenograms of the chest were normal. She was started on penicillin at a dose of 100,000 units every 3 hours, plus some supportive measures. This was supplemented 2 weeks later by 300,000 units of crysticillin twice a day. On March 24, 1951, the osteomyelitic cavity was unroofed and packed. Smears of the exudate showed filaments with beaded Gram-positive granules. Culture of the same material revealed an aerobic actinomyces later identified as *N. asteroides*. The organism was moderately sensitive to terramycin, slightly sensitive to neomycin, and resistant to all other available antibiotics. On three other occasions (March 29, March 30, and April 4, 1951) the same organism was isolated from exudate from the right thigh and in the last specimen *Staphylococcus aureus* and hemolytic *Escherichia coli* also were isolated. Three days after the operation, the penicillin was increased to 200,000 units every 3 hours. Four days after unroofing the osteomyelitic cavity, a fluctuant swelling of the distal phalanx of the right middle finger developed. This was incised and drained the following day. Throughout the patient's stay in the ward, her temperature was remittent, ranging from 100° to 103.5° F., being highest toward the evening. She appeared toxic, with continuous draining of the wound in her right thigh. Gantrisin was started on April 1, 1951, with a dose of 1.0 gm. four times a day. About this time, stiffness of the neck became manifest. Two days prior to her death, she developed moderate acidosis, and 2,000 cc. of 1/6 molar lactate solution was given. Penicillin was stopped and terramycin was started with a dose of 1.0 gm. every 4 hours. She also received several blood transfusions. She developed diarrhea and died at 10:07 p.m., April 7, 1951.

Necropsy

Necropsy was done 17 hours post mortem. The body was markedly emaciated, weighing 40 kg., with a body length of 150 cm. The mucous membrane of the mouth and pharynx was covered with a violaceous substance and the whitish plaques described during life could not be discerned. There was a 12.0 cm. incision at the lateral surface of the upper third of the right thigh. The incision opened into an abscess cavity involving the underlying bone and containing about 30.0 cc. of moderately thick, reddish brown, odorless exudate. The upper third of the femur showed extensive necrosis in an area approximately 4.0 cm. square and involving about one-third of its circumference. There was extension of the abscess cavity into the medial aspect of the thigh through the anterior surface of the femur.

The heart (Fig. 1) showed extensive necrosis of a papillary muscle of the mitral valve and its corresponding chorda tendinea, with several sessile vegetations adherent to its surface. The involved papillary muscle was yellowish gray. Section through this papillary muscle

showed three small abscesses ranging from 0.1 to 0.3 cm. in diameter and containing greenish yellow, thick, odorless pus.

The left kidney was hydronephrotic, weighing only 60 gm. after the fluid content was drained. The right kidney weighed 255 gm. and revealed three irregular, elevated patches with a yellowish, glistening, central portion and a reddish purple periphery (Fig. 2). These patches, which were typical of early infarcts of the kidney, ranged from 1.25 to 2.5 cm. in diameter. Hemisection through the right kidney revealed numerous small, yellowish abscesses in the cortical portion ranging from 0.1 to 0.2 cm. in diameter. The left ureter showed a point of obstruction about 2.0 cm. proximal to the bladder (probably secondary to a previous hysterectomy).

Sections through the right adrenal gland revealed multiple abscesses, 0.3 to 0.5 cm. in diameter, containing a moderate amount of greenish yellow, thick, odorless pus. The left adrenal gland was normal.

In the brain, a small abscess was found in the posterior tip of the left occipital lobe. The abscess was approximately 1.0 cm. in diameter, was encapsulated, and contained a moderate amount of greenish yellow pus. A small grayish green area of softening, measuring about 2.0 cm. in diameter, was found in the anterior portion of the left corpus callosum.

The mesenteric and para-aortic lymph nodes were discretely enlarged but showed no abscess formation. The lungs, spleen, gastrointestinal tract, pancreas, liver, and bladder showed nothing abnormal.

Histologic Study

The histologic changes in the organs showing abscesses grossly, namely, the papillary muscle of the mitral valve, the right kidney, the right adrenal gland, the right femur, and the brain, were similar in all respects. The main feature was extensive caseation necrosis surrounded by a wall of monocytes, lymphocytes, and plasma cells (Fig. 3). Giant cells were not seen and fibroblastic reaction did not occur. The central portion of these necrotic areas was composed of cellular débris and degenerating leukocytes. In addition, microscopic abscesses were seen in the enlarged mesenteric and para-aortic lymph nodes. No organisms were seen with ordinary hematoxylin and eosin staining. Using the MacCallum-Goodpasture special stain, however, numerous Gram-positive, slender, filamentous forms with clusters of Gram-negative coccoid-like bodies were seen within these abscesses (Fig. 4). Routine Ziehl-Neelsen technic showed numerous weakly to moderately acid-fast, slender, branching filaments and granules within these necrotic areas. The McManus-Hotchkiss stain failed to show organisms

in the tissues studied except in the abscess cavities in the brain where reddish filaments and granules were found in a greenish background. A section through the greenish gray area of softening in the anterior portion of the left corpus callosum showed numerous micro-abscesses containing many organisms.

In addition to the abscesses, the right kidney showed extensive recent septic infarcts with organism-laden thrombi within some blood vessels. The sections through the right femur showed extensive destruction of bone with a moderate amount of granulation tissue. The same organism seen in the abscesses in the other organs was present. In one field there was a small area of caseation necrosis like those seen elsewhere. There were no significant findings in the lungs except a mild bronchopneumonia of the right lower lobe which failed to show the characteristic slender filaments even after special staining.

Bacteriologic Studies

Post-mortem bacteriologic studies revealed abundant *N. asteroides* in the specimens obtained from the right adrenal gland, papillary muscle of the mitral valve, and brain abscess.

The smear of the pus from the right femur obtained surgically showed branching filaments beaded with Gram-positive granules, which were suggestive of an actinomyces. However, culture of this material yielded in 48 hours an abundant growth of flat, fluffy, white colonies of leathery consistency on both aerobic and anaerobic plates (Fig. 6).

Subsequently, the same organism was cultured from pus collected from the felon when it was incised and from the right adrenal gland, endocardial vegetations, and brain abscesses at necropsy. A culture from the femoral lesion at time of wound dressing, 6 days postoperative, was negative, but occasional beaded filaments were seen in the direct smear. By this time, however, the wound had become secondarily contaminated with *Escherichia freundii* and the failure to grow the earlier organism could be explained on the basis of competition with a more rapidly growing bacterium. In a third specimen, 12 days postoperative, the filamentous strands could still be seen, but a culture yielded only *Aerobacter aerogenes, E. coli,* and *Staph. aureus.*

Further studies indicated the organism to be *N. asteroides.* Microscopically, the organism appeared as Gram-negative, branching filaments studded with tiny, Gram-positive-staining granules. Occasionally, filaments or sections thereof were strongly Gram-positive. The freshly isolated subculture was partially acid-fast in that some of the bacillary forms, broken away from the mycelium, retained the fuchsin while the bulk of the growth took only the counterstain. A wet mount

of an entire colony from a broth culture showed irregular branching aerial hyphae, many of which contained highly refractile granules (Fig. 5).

The surface colonies on blood, Sabouraud's, Czapek's, nutrient agar, and Petragnagni's media were very similar. After 1 to 2 weeks of aerobic incubation at 37° C. they were 2 to 3 mm. in diameter, snowy white with a matte surface, tough, and adherent. Prolonged incubation (4 to 6 weeks) brought out some changes in pigmentation. On Sabouraud's medium the portion of the colony next to the agar became bright orange. The pigmentation on Petragnagni's medium was very similar, but in addition the white mycelium developed a light coral hue. By contrast, the growth on Czapek's medium retained its chalky white appearance. In agar shake cultures the deep growth appeared as white cotton ball colonies; similarly, in brain-heart-infusion broth they grew in clusters of compact, fluffy, cotton ball colonies. Gelatin was not liquefied and milk was unchanged, even though the organism grew well in both media.

This particular strain of *N. asteroides* posed a problem in identification because of its failure to produce yellow or orange pigmentation on primary isolation. The organism was described by Conant and Rosebury[40] as being glabrous, rarely chalky. Bergey's Manual[41] also mentioned that occasional variants are chalky white. In reviewing the literature, 9 different authors have described organisms that do not differ materially from the present culture.[4,7,9,10,15,25,28,36,42]

Inasmuch as there are varying reports about the pathogenicity of this species, infectivity tests were made on mice, guinea-pigs, and rabbits. The mice and guinea-pigs were injected intraperitoneally with 0.5 ml. of broth culture and observed for a period of 1 month, during which time they remained active and showed no evidence of disease. Similarly, a rabbit which was injected intravenously with 1.0 ml. of broth culture failed to develop any sign of illness.*

Sensitivity tests by the disk method showed *N. asteroides* (Fig. 7) to be moderately sensitive to terramycin, slightly sensitive to neomycin, and resistant to penicillin, streptomycin, aureomycin, chloromycetin, and polymyxin. Subsequently, a tube test indicated the strain to be sensitive to 1.0 μg. per ml. of terramycin.

Discussion

The case of nocardiosis reported in this paper presented several significant features. In the first place, of the 40 or more case reports

* However, 6 months after the preparation of this manuscript one of the mice developed a subcutaneous abscess in the neck which at necropsy was found to contain a caseous exudate; there was also a hepatic abscess containing caseous material from which *N. asteroides* was cultured.

in the literature, this is the only case in which the primary focus of infection was femoral osteomyelitis. It is also one of the few cases in which involvement of the lungs was absent. The involvement of the heart also made this case interesting inasmuch as the clinical signs produced thereby suggested the possibility of bacterial endocarditis. The finding of moderate sensitivity of the organism to terramycin *in vitro* opens a new field in therapeutic approach, although delay in the institution of specific therapy prevented an adequate trial. Gordon and Hagan[39] isolated several species of Nocardia from soil, which were culturally related to organisms isolated from known human cases and which produced lesions in rabbits not distinguishable from those produced by known pathogenic types. This finding suggests that the organisms may gain access to the blood stream through superficial breaks in the skin and mucous membrane.

The mouth lesions in this case developed after the femoral lesion and thus the oral mucosa cannot be considered a portal of entry. Whether the initial localization was in the heart or femur cannot be stated definitely. The long interval between the discovery of the femoral lesion and death would suggest that the initial infection had occurred in the bone with subsequent cardiac involvement and then hematogenous spread to other organs.

Summary

In a fatal case of nocardiosis occurring in a colored female, 43 years old, the initial infection was an osteomyelitis of the right femur with subsequent embolic abscesses in the heart, brain, kidney, adrenal gland, and mesenteric and para-aortic lymph nodes.

The organism showed a moderate sensitivity to terramycin and a slight sensitivity to neomycin but neither of these drugs received a satisfactory therapeutic trial. Their value in cases of nocardial infection should receive further study.

We are indebted to the Misses Alice Kester and C. Burr McDonald for assistance in identifying the organism, to Mr. L. Soneson for the photomicrographs, and to Dr. A. Reynolds Crane for his advice and criticism.

ADDENDUM

After this paper had been accepted for publication, another report[43] on nocardiosis appeared, adding 3 more cases to the literature. The first case was a white male, 39 years old, with silicosis, who developed multiple subcutaneous abscesses which were positive for *Nocardia asteroides*. Necropsy revealed nocardial bronchopneumonia coexisting with anthracosilicosis, subcutaneous and renal abscesses, meningitis, and multiple cerebral and cerebellar abscesses. The organism was demonstrated in the brain, lungs, and kidneys. The second case was a white male, 60 years of age, who was admitted for cardiac failure. Necropsy showed right hydrothorax, right-sided bronchopneumonia, cerebral abscesses, and a left axillary abscess. Culture obtained post mortem from the pleural fluid, left lateral ventricle, and occipital abscess were

positive for *N. asteroides*. The third case was a white female, 23 years old, who was admitted for shortness of breath, orthopnea, night sweats, and hemoptysis. Left-sided hydrothorax was found and culture of the pleural fluid revealed the causative organism. The patient recovered after 2 years with combined streptomycin and penicillin therapy. Organisms from the first case were slightly sensitive to a combination of penicillin and streptomycin *in vitro*.

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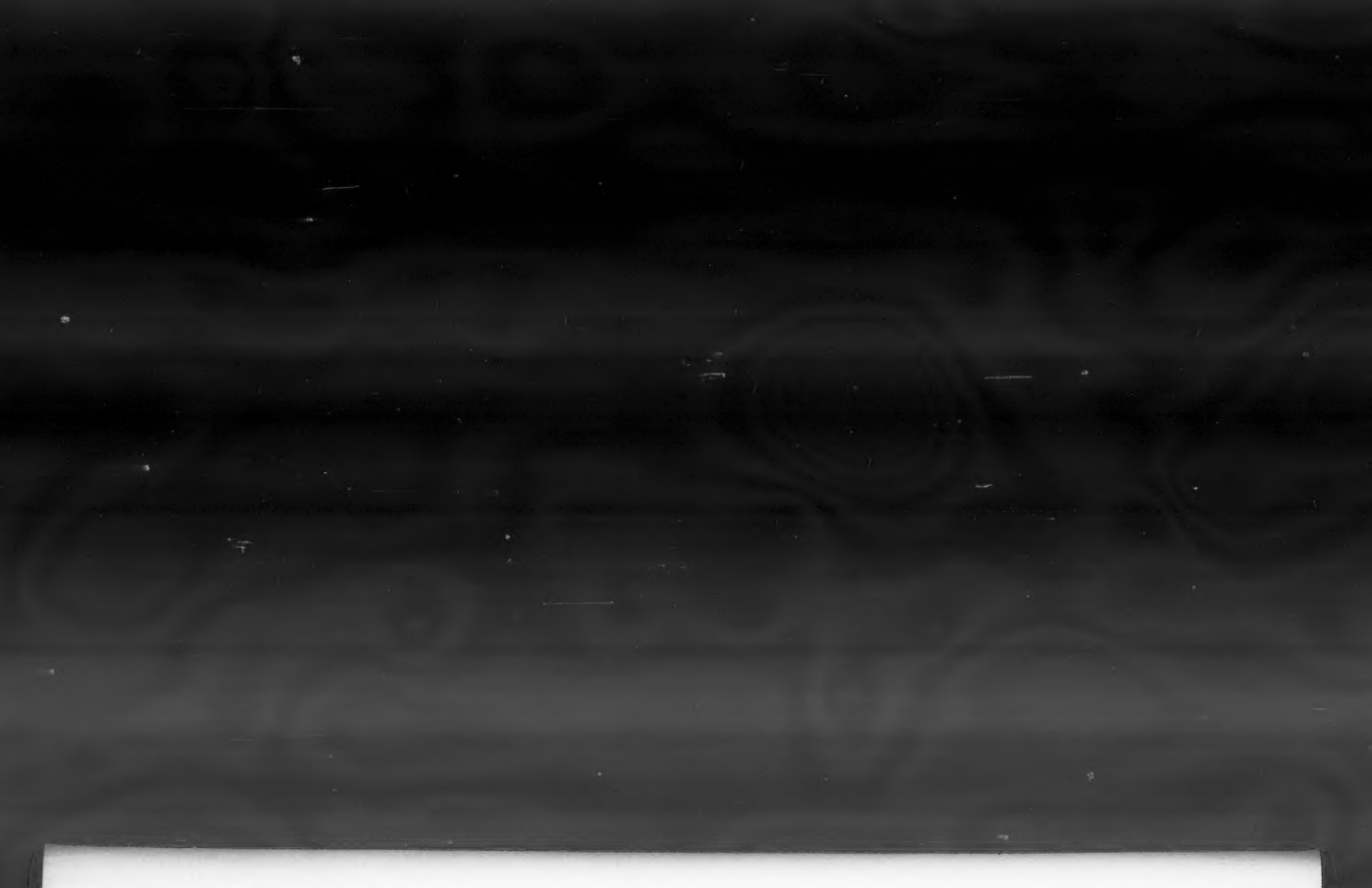
[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 85

FIG. 1. Heart showing necrosis and abscess formation of a papillary muscle of the mitral valve.

FIG. 2. Right kidney showing multiple septic infarcts.



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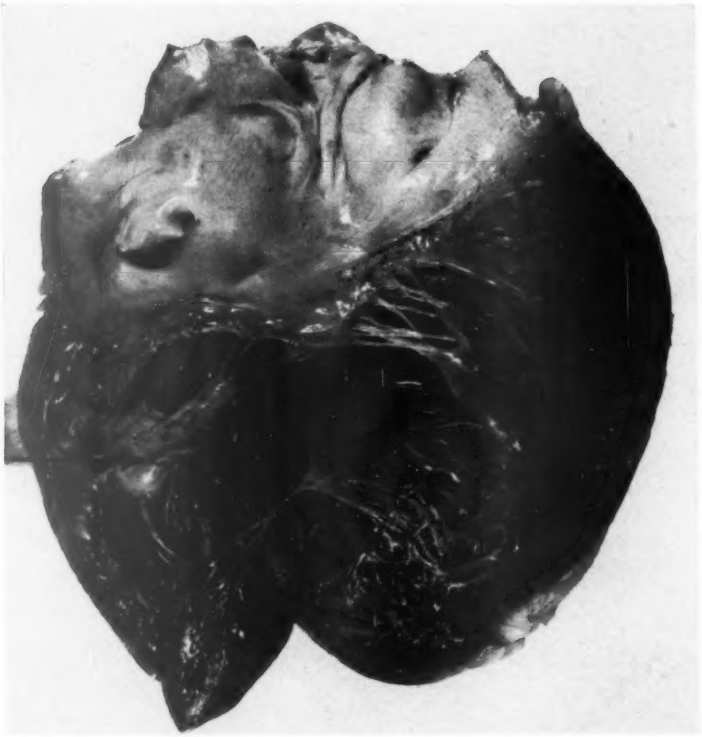

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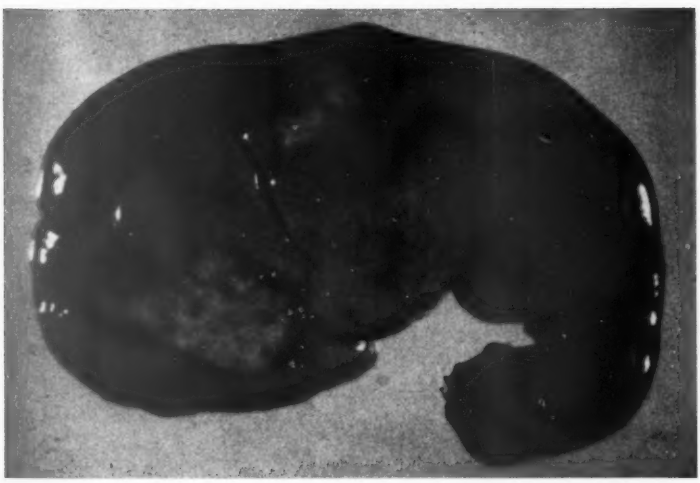
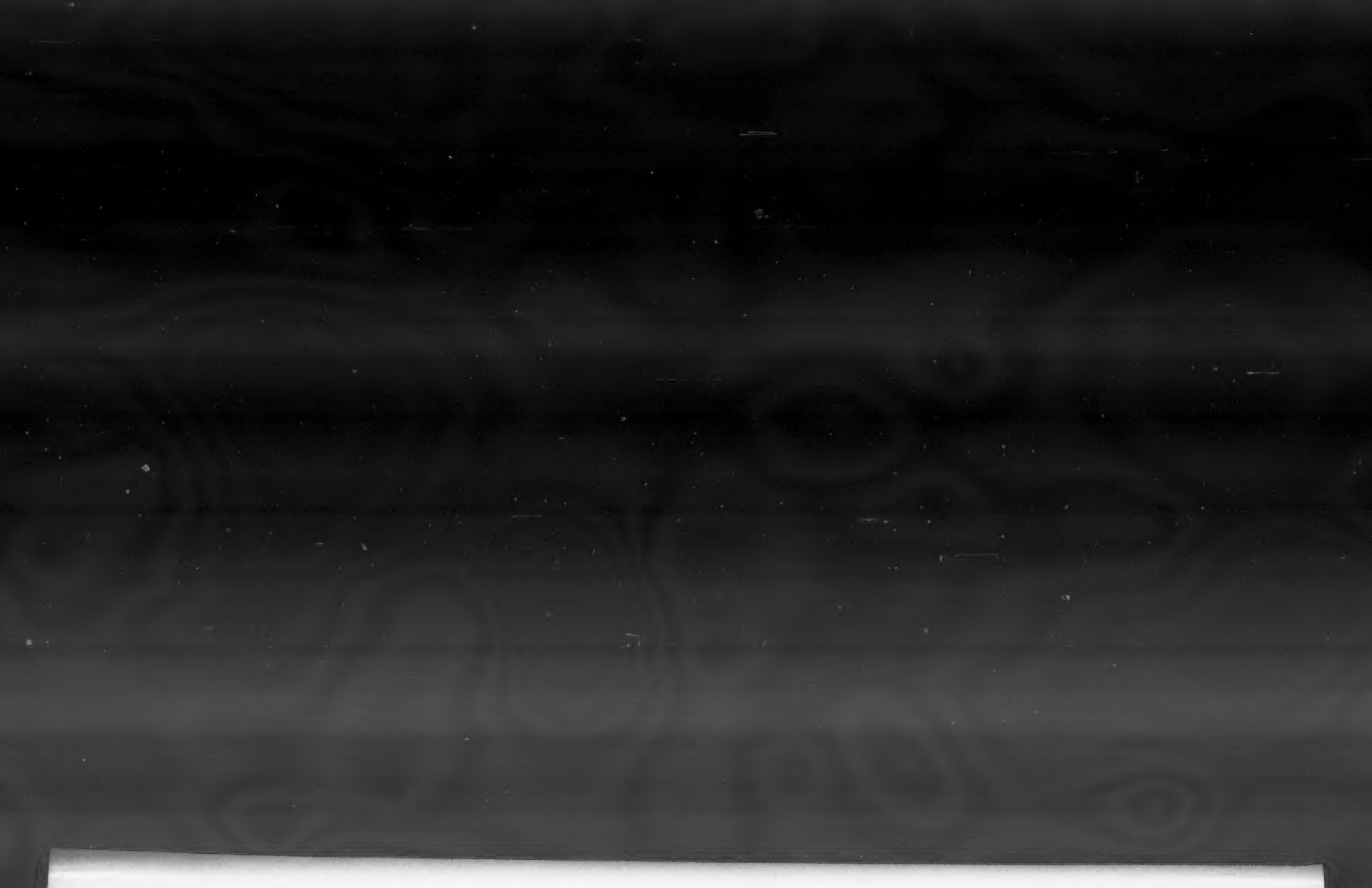


PLATE 86

FIG. 3. Necrotic area in the papillary muscle surrounded by a wall of lymphocytes, monocytes, and plasma cells. Hematoxylin and eosin stain. × 450.

FIG. 4. The same area in the papillary muscle under oil immersion showing numerous fragments of *Nocardia asteroides*. MacCallum-Goodpasture's stain. × 1000.



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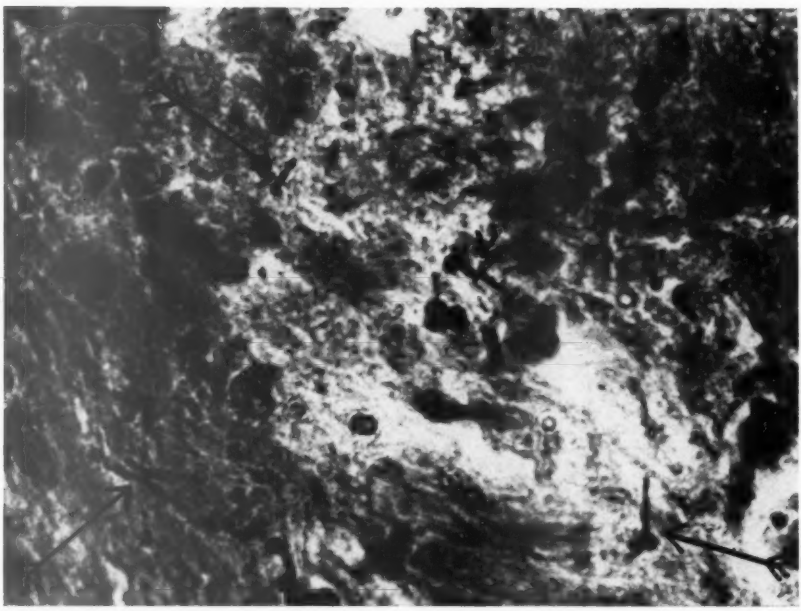


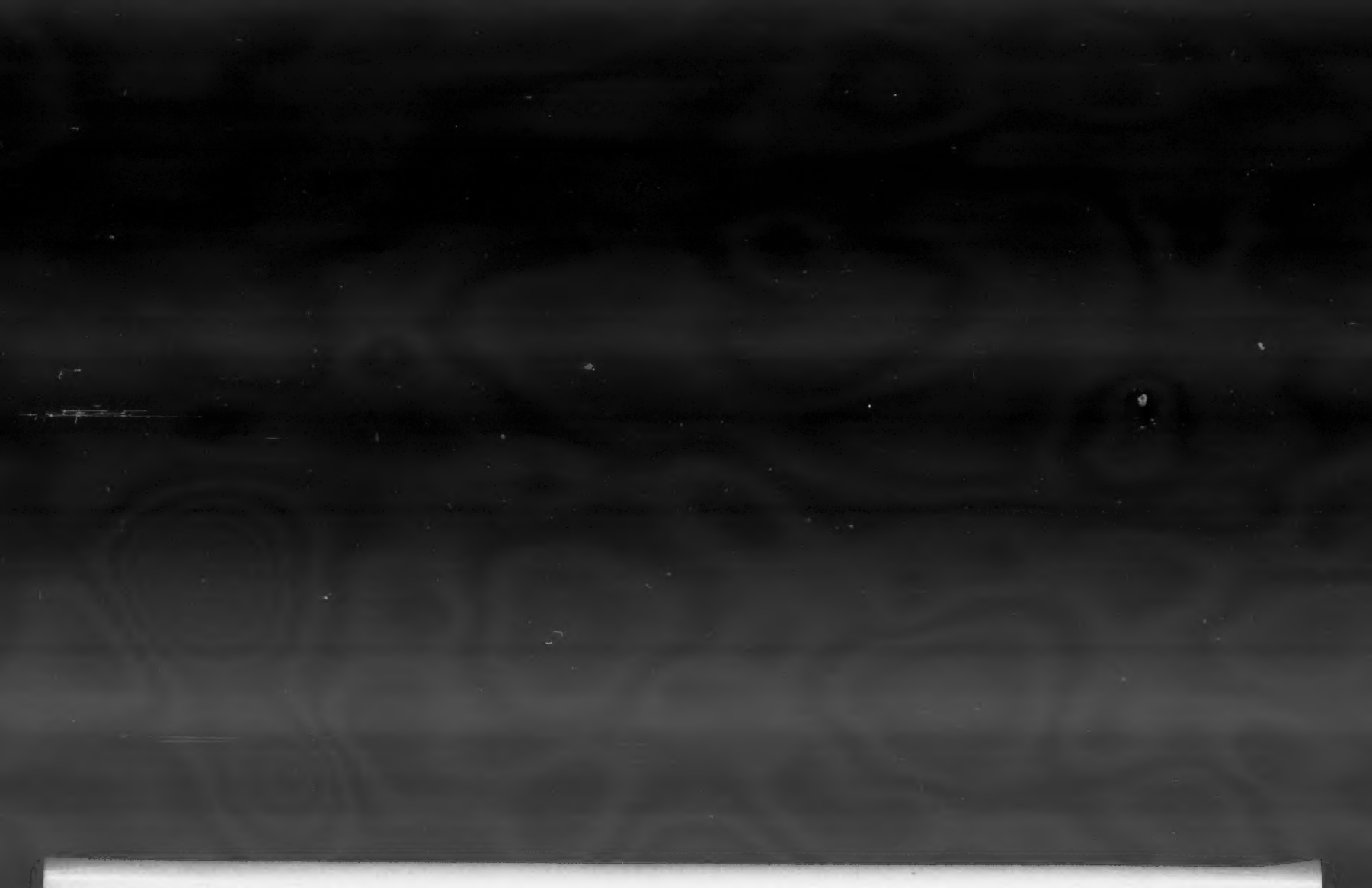
Cruz and Clancy Nocardiosis

PLATE 87

FIG. 5. Marginal filaments from a colony of *N. asteroides* from broth culture. × 1000.

FIG. 6. Blood agar plate showing primary isolation of *N. asteroides* from the femur. × ¾.

FIG. 7. Sensitivity test. Reading clockwise starting at **X**: penicillin, terramycin, neomycin, chloromycetin, aureomycin, streptomycin, and, center, polymyxin. There is inhibition of growth with terramycin and neomycin. × ¾.



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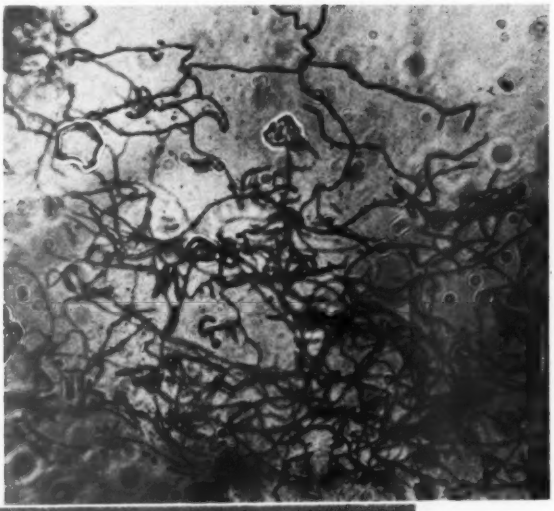

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7

THE HISTOLOGY OF ANTIGENICALLY STIMULATED LYMPH NODES IN RABBITS GIVEN ACTH OR CORTISONE *

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The reduction in the mass of lymphoid tissue after administration of active adrenocortical substances or of adrenocorticotropic hormone in animals with intact adrenal glands is now a well established phenomenon.[1-5] This is accompanied by a decrease in the number of circulating lymphocytes,[6-8] and is coincident with dissolution of the small adult lymphocytes in the thymus, spleen, bone marrow, and lymph nodes.[2,5] The formation of reaction centers, the swelling of the sinusoidal reticulum cells, and the inhibition of follicular mitosis also have been reported to occur in lymph nodes of animals given adrenocortical substances.[2]

In adrenalectomized animals, on the other hand, an increased mass of lymphoid tissue has been reported to develop, especially in the visceral lymph nodes.[9-11] Here, the perifollicular element, especially, is widened by increased numbers of large and small cells.

The location of the site of production of antibodies in lymphoid structures appears to be well established[12-17] by the demonstration, early in the period of response, of increased antibody titers within lymph nodes and in the efferent lymph from the regional lymph nodes in comparison with that in the serum. The observation that antibody formation is suppressed by the use of agents such as radiation or nitrogen mustards, which are known to destroy lymphoid tissue,[18-21] also appears to implicate the lymphoid tissue in antibody production.

The cell or cells within the lymphoid tissue that are responsible for the production of antibodies have not been clearly defined. Through the use of various technics, studies purporting to establish the lymphocytes, transitional or reticular cells, or the adult plasma cells as sources of antibodies have presented conflicting conclusions. There is little evidence that the macrophages produce antibodies, although their activity as digesters and preparators of foreign protein cannot be denied.[22,23] Most authors agree that the transitional cell (reticulum cell, immature lymphocyte or preplasma cell, or preplasmablast) plays an important, and perhaps a major, rôle in the formation of antibody protein.[24-26] Others point out that the lymphocytes also are intimately related to the antibody response,[12-14] though the exact connection is not established.[27]

* Received for publication, February 11, 1952.

Adrenocorticotropic hormone and cortisone have been shown by the majority of workers to inhibit partially the formation of antibodies.[28-33] However, their results, with the exception of those reported by Germuth and his associates,[30] have not been clear-cut. On the other hand, Chase, White, and Dougherty[34] reported that an increased titer of antibody was obtained by the simultaneous administration of antigen and adrenocortical extract.

With this background in mind, experiments designed to demonstrate the histologic effects of adrenocorticotropic and adrenocortical hormones in antigenically stimulated lymph nodes of rabbits were carried out. In order to determine the sequence of changes in control and hormone-treated animals during the period of maximum antigenic stimulation and antibody production, a single injection of antigen was given in an area from which the lymphatic drainage would pass primarily to a single lymph node.[35] It was hoped that this method might give some insight into the cellular source of antibody and the histologic manifestations of adrenocortical inhibition of antibody formation.

Materials and Methods

New Zealand white rabbits weighing between 1.4 and 2.0 kg. were used. The animals were maintained in separate cages on a diet of purina chow, supplemented with fresh carrots. After a short period of observation, usually about 1 week, one or more leukocyte and differential counts were made, using freely flowing blood from an incised vein in the ear. A control sample of blood was collected aseptically by cardiac puncture for antibody determination. Then 0.5 cc. of *Salmonella typhi* "O" antigen,* which had been washed three times and restored to its original volume with saline solution, was injected into the right foot pad. The animals were divided into three major groups of 12 animals each. The animals in group 1 were killed 48 hours after injection of the antigen, those in group 2 were killed 96 hours after injection, and those in group 3 were killed 8 days after injection of the antigen. These major groups were further divided into three equal subgroups: one consisted of 4 antigen-treated control animals, one consisted of 4 antigen-treated animals given daily doses of ACTH, and the third consisted of 4 antigen-treated animals given daily doses of cortisone. In group 2, 5 rather than 4 antigen-treated control animals were used.

Serial total and differential leukocyte counts were made at intervals during the experimental period. At the time the animal was killed by intracardiac injection of air, a second sample of serum was obtained.

* Lederle febrile typhoid "O" antigen, product no. 2483.

In group 3, carried for 8 days after the antigen was administered, an additional sample of serum was obtained 4 days after the initial injection of antigen.

The animals receiving ACTH were given the equivalent of 30 mg. of the standard of LA 1 A Armour adrenocorticotropic hormone each day. In group 1, this was divided into four equal portions and administered at 6-hour intervals. In groups 2 and 3, it was divided into three equal portions and administered at 8-hour intervals. The first dose was given at the time of injection of antigen, intramuscularly, into the left thigh. The cortisone (17-hydroxy-11 dehydrocorticosterone—Merck) was given intramuscularly once a day in a dose of 4 mg. The first dose was injected 4 hours before the administration of the antigen. An additional milligram was given 6 hours before the animal was killed.

Immediately after death, the subcutaneous tissue of the foot pad and the interosseous muscles were dissected from both the injected and uninjected sides. The popliteal lymph nodes were removed, dissected free of adherent fat, weighed to the nearest milligram, divided, and placed with the foot pads in Zenker's acetic acid solution and in absolute alcohol. The adrenal glands, liver, and spleen were weighed. Paraffin sections of Zenker-fixed tissue were stained with toluidine blue and eosin, and the alcohol-fixed nodal tissue was stained by Taft's modification of the methyl-green-pyronine stain.[36] Serum antibody titers were determined against unwashed typhoid "O" antigen (Lederle), according to the microscopic slide technic of Welch and Stuart.[37] The popliteal lymph node from the uninjected side in each animal served as an additional control, since it received only minimal stimulation from the injected antigen, but received the full effect of the adrenocorticotropic and adrenocortical hormones.

Wherever possible, a quantification of histologic features was attempted. Measurements were made or values of 1 to 4 were assigned arbitrarily to the various nodal constituents. For example, the width of the cortex of a node was measured in high-power field diameters in several quadrants. The size of the germinal follicles was determined similarly; all of the follicles within a given section were measured by their high-power field diameters, and the result was expressed as a mean number. This method was found to be reproducible. The predominance of one cell type was estimated by its relative frequency and was assigned a value of from 0 to 4. Thus, an appreciable number of cells of one kind which made up less than 25 per cent of the total number of cells was assigned a value of 1, and if a given type of cell made up 75 to 100 per cent of the total number of cells, it was assigned a value of 4. The number of mitotic figures present in a high-power

field similarly was assigned an arbitrary value: 1 indicated the presence of 1 to 5 mitotic figures; 2, 5 to 10; 3, 11 to 20; 4, more than 20.

The changes observed in the popliteal lymph nodes of the control rabbits after the injection of typhoid antigen were similar to those reported by Ehrich and Harris.[13] After 2 days, a diffuse polymorphonuclear reaction was present in the perinodal structures, and the cortex was greatly widened (Figs. 1 and 3) and contained a variety of small lymphocytes, large lymphocytes, swollen reticular cells (showing an occasional mitotic figure), and eosinophilic polymorphonuclear cells. Germinal follicles were present, but were of moderate size and indefinite outline. Many of these contained reaction centers filled with polymorphonuclear cells and débris. No secondary follicles were seen, and no mitotic figures were observed in the germinal follicles, although they could be found in the cortex. The medullary cords were swollen and contained a variety of cells, some in mitosis. The sinusoids were swollen, and the macrophages contained a few polymorphonuclear eosinophils and considerable nuclear débris. After 4 days, the infiltration of the perinodal tissue was decreased, and the cortex was very wide and contained sheets of adult and immature lymphocytes, as well as large germinal follicles with active secondary follicles, having many mitotic figures. The peripheral and central sinusoids contained a few eosinophils and a moderate number of large and small lymphocytes. The medullary cords (Fig. 5) were swollen and contained a predominance of the transitional or preplasma cells, but also numbers of eosinophils and lymphocytes. The sinusoidal macrophages were less swollen, but still contained much débris. After 8 days, the perinodal tissues still contained a few chronic inflammatory cells, but the peripheral sinuses often were empty. The cortex was approaching normal width, but contained an increased number of small follicles with still active secondary follicles. The cortex was comparatively uniform in cell content with many adult lymphocytes. The medullary cords were less wide and still contained a high proportion of large transitional cells and an increasing proportion of small lymphocytes. The macrophages in the central sinusoids approached their inactive state, but the sinusoidal spaces contained moderate numbers of both large and small lymphocytes.

The distribution of the pyronine-positive areas, as revealed by the methyl-green-pyronine stain, was observed to shift considerably during the course of the experiment in the antigen-injected controls. In the nodes of the control animals killed 2 days after injection of the antigen, the pyronine-staining material was present in single cells in the middle and inner cortical layers and in a larger percentage of cells in the medullary cords, while none was observed in the reaction centers or in

the sinusoids. After 4 days, the largest mass of pyronine-positive cells was in the germinal follicles (Fig. 9), and the cytoplasm here was stained intensely. There were single scattered pyronine-positive cells outside the germinal follicles in the cortex. In the medullary cords, the cytoplasm of the pyronine-positive cells was more abundant, but the intensity of the reaction did not differ from that within the germinal follicles. These medullary cells continued to have positively stained cytoplasm even while in mitotic division. The reticulo-endothelial macrophages took a faintly positive stain. After 8 days, the pyronine-positive material in the secondary germinal centers was sharply reduced in amount and intensity. Likewise, the number of the positive cells in the cortex was greatly reduced. Only a minimal amount of reactive material was observed in the medullary cords. The macrophages did not stain at 8 days.

Two days after injection of the antigen, the foot pads showed definite necrosis of muscle, exudation of polymorphonuclear cells, and deposition of fibrin in the loose interstitial areolar tissue. After 4 days, early repair was in progress with sarcolemmal nuclear proliferation and proliferation of fibroblasts and mononuclear cells at the edges of the largely necrotic mass of polymorphonuclear cells. Some peripheral organization of the exudative débris and proliferation of lymphocytes had occurred. After 8 days, small nodules of lymphocytes were present, and the edge of the exudative mass was invaded by proliferating fibroblasts and mononuclear cells. The observed proliferation of sarcolemmal sheath cells continued.

Results

Group 1 Animals Killed Forty-eight Hours after Injection of Antigen

In group 1 there was considerable reduction in the weights of the popliteal lymph nodes on both injected and uninjected sides in the animals receiving ACTH or cortisone (Table I). The spleens were smaller in the animals receiving ACTH or cortisone than they were in the controls. The adrenal glands were larger in the animals treated with ACTH, but smaller in those treated with cortisone than they were in the controls. No demonstrable antibody was found in the serum of any animal in group 1.

The nodes on the antigenically stimulated side were less congested in the animals treated with ACTH or cortisone than they were in the control animals. No gross or microscopic differences were found between the foot pads of the control group and those of the ACTH-treated animals. The foot pads of the cortisone-treated animals were not grossly congested, as were those of the other two groups. Micro-

TABLE I

Group 1: Animals Killed Forty-eight Hours after Injection of Antigen

Rabbits	Weight of right adrenal gland		Weight of spleen		Weight of liver		Right popliteal lymph node												Left popliteal lymph node											
							Weight		Width of cortex		Diameter of germinal follicles		Outline of germinal follicles		Size and number of reaction centers		Size of medullary cord		Weight		Width of cortex		Diameter of germinal follicles		Outline of germinal follicles		Size and number of reaction centers		Size of medullary cord	
	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	*	*	*	*	*	*	*	*	*	*	gm.	gm.	*	*	*	*	*	*	*	*	*	*
Control	.089	.070 to .114	1.76	1.00 to 3.43	124	75 to 176	.584	.367 to .737	2.7	2.5 to 3.0	1.2	0.5 to 2.0	1	± to 2	2	0 to 3	2	1 to 4	.245	.156 to .297	0.5	0.5 to 0.7	0.4	0.4 to 0.5	3	2 to 3	0	0	±	
Received ACTH	.139	.107 to .180	.951	.827 to 1.007	92	66 to 131	.309	.240 to .369	1.4	1.3 to 1.5	0.2	0 to 0.5	0	0	±	0 to ±	1	1 to 2	.151	.121 to .209	1.0	0.5 to 2.0	0.5	0.5 to 0.6	2	± to 3	0	0	1	1
Received cortisone	.075	.071 to .081	.685	.637 to .727	108	110 to 127	.298	.232 to .400	1.2	1.1 to 1.4	0.8	0.4 to 1.0	1	± to 2	0	0	1	1 to 2	.160	.094 to .280	1.2	1.0 to 1.6	0.6	0.4 to 0.8	2	1 to 3	±		0	0

TABLE II

Group 2: Animals Killed Ninety-six Hours after Injection of Antigen

Rabbits	Weight of right adrenal gland		Serum titer		Right popliteal lymph node														Left popliteal lymph node											
					Weight		Width of cortex		Diameter of germinal follicles		Diameter of secondary follicles		Size and number of reaction centers		Follicular mitotic figures		Prominence of medullary transitional cells		Weight		Width of cortex		Diameter of germinal follicles		Diameter of secondary follicles		Size and number of reaction centers		Follicular mitotic figures		Prominence of medullary transitional cells	
	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range
	gm.	gm.			gm.	gm.	*	*	*	*	*	*	*	*	*	*	*	*	gm.	gm.	*	*	*	*	*	*	*	*	*	*	*	*
Control	.101	.052 to .124	1:170	1:4 to 1:320	.646	.441 to .855	2.1	1.0 to 3.0	1.1	0.7 to 1.5	0.5	0 to 1.0	1	0 to 2	3	1 to 4	1	1 to 2	.199	.112 to .250	0.9	0.3 to 1.8	0.7	0.6 to 0.9	0.3	0 to 0.7	±	0 to 2	1	0 to 2	±	0 to 2
Received ACTH	.088	.072 to .132	1:17	1:8 to 1:20	.395	.240 to .595	1.4	1.0 to 2.0	0.8	0 to 1.6	0.2	0 to 0.3	±	0 to 1	±	± to 1	3	3 to 4	.162	.120 to .267	1.0	0.7 to 1.5	0.6	0.4 to 0.8	0.1	0 to 0.3	±	0 to 1	0	0	1	0 to 3
Received cortisone	.082	.062 to 1.0	1:100	1:80 to 1:160	.281	.178 to .340	1.5	1.4 to 1.7	0.7	0 to 1.0	0.3	0 to 0.4	0	0	±	0 to 2	3	2 to 3	.137	.090 to .192	0.6	0 to 1.0	0.2	0 to 0.5	0.4	0 to 0.5	±	0 to ±	0	0	1	0 to 2

* For quantitative values other than those in the metric system, see page 631.

scopically, after 2 days, the perinodal areolar tissue of the animals which received ACTH contained large masses of infiltrating cells, while that of the cortisone-treated and control animals showed only minimal to moderate reactions. Fibrin plugs were present in the afferent lymphatics of 3 of the 4 ACTH-treated animals, but were not present in any of the cortisone-treated or control animals. Similarly, the peripheral sinusoids of the ACTH-treated animals, although only slightly more dilated, appeared to contain a larger number of cells, both eosinophils and large transitional cells; all groups contained moderate numbers of lymphocytes. The fixed reticulo-endothelial cells of the peripheral sinuses displayed little change in any group.

Microscopically, the cortices of the lymph nodes were much narrower in the animals treated with ACTH or cortisone than they were in the antigen-injected controls (Figs. 1, 2, 3, and 4). They were also qualitatively different in the following respects: (1) Throughout the cortex in the ACTH-treated animals, and extending into the medullary cords, were numerous pyknotic lymphocytes. These often occurred in sheets, but it was difficult to recognize lymphocytes beyond the stage of pyknosis. The loss of small lymphocytes in the cortex uncovered the fixed structural reticular cells in focal areas of the node. Occasionally, small foci of pyknotic lymphocytes were seen also in the controls, away from the reaction centers, but these were inconspicuous as compared to those in the ACTH-treated animals; pyknotic, small lymphocytes were even less conspicuous in the cortisone-treated animals. (2) The outlines of the few remaining follicles in the ACTH-treated animals were less distinct than they were in the antigen-injected controls, there being a less dense rim of adult lymphocytes about the follicles. This comparative obliteration of follicular outline was less well marked in the cortisone treated animals. (3) The germinal follicles in the cortex, where discernible, were extremely small in contrast to those in the control animals. In none of the groups were secondary centers of active lymphoblastic proliferation still discernible. (4) Extremely small reaction centers, consisting of a mass of polymorphonuclear cells, disintegrating lymphocytes, and nuclear débris, were present in the cortex in lymph nodes from 3 of the 4 ACTH-treated and in one of the 4 cortisone-treated animals. Three of the 4 control animals had large and conspicuous reaction centers in the lymph node cortices. (5) No significant mitotic activity was apparent in the germinal follicles of either treated group, but in the control animals, occasional mitotic figures were apparent in the greatly thickened cortex. (6) The medullary cords of the lymph nodes in the animals treated with ACTH or cortisone were narrow and inconspicuous, but their cell content did

* For quantitative values other than those in the metric system, see page 631.

not differ qualitatively from that of the control animals. (7) The appearance of the intermediate and central sinuses of the lymph nodes in animals treated with ACTH or cortisone was striking because of the swelling of the sinusoidal macrophages, which choked and obliterated the sinusoidal lumen. Large numbers of intact, eosinophilic polymorphonuclear cells were present in the sinuses and within the cytoplasm of the macrophages. The processes of digestion of the phagocytized débris appeared to be less advanced than in the untreated animals. In the latter, the macrophages only rarely filled the sinusoids, the eosinophils were reduced in number, and the cytoplasm of the phagocytes rarely contained more than basophilic débris.

With the methyl-green-pyronine stain, the cytoplasm of some individual cells in the cortex and medullary cords of lymph nodes from animals treated with ACTH and cortisone stained more intensely than that of their neighbors, but there were fewer affected cells than there were in the controls.

At the end of 2 days, the differences between the three groups in the histologic features of the popliteal nodes from the sides not antigenically stimulated were much less conspicuous. The width of the cortex was nearly the same, and in outline and size the follicles were nearly equal. Small secondary follicles with a few mitotic figures were present in 2 of 4 animals in each group of the hormone-treated rabbits. In animals observed for 4 or 8 days, the histologic similarity of the lymph nodes not antigenically stimulated was even more apparent in all three groups.

Group 2 Animals Killed Ninety-six Hours after Injection of Antigen

In 3 of the 4 ACTH-treated animals and in all of the cortisone-treated animals in group 2, the popliteal lymph nodes draining the right foot pad were smaller than those in the control animals (Table II). In the ACTH-treated animals, the serum antibody titers were lower than they were in the controls, with one exception. In the cortisone-treated animals, the serum antibody titers were slightly, but not significantly, lower than those in the controls. No significant differences in the perinodal tissue could be discovered between the groups. The foot pads of the animals treated with ACTH and cortisone showed less proliferation of granulation tissue and a decided reduction in the number of lymphocytes in the inflammatory reaction about the granulation tissue.

Microscopically, the lymph nodes of the hormone-treated animals had thinner cortices (Table II), but, like those of the controls, they contained sheets of small lymphocytes. The germinal follicles of the hormone-treated animals also were smaller, although the outline of the

germinal centers was distinct. This finding was in contrast to the poorly outlined centers in the animals killed 48 hours after injection of antigen. The reaction centers in the treated animals were considerably reduced in area and number, in contrast to the persisting reaction centers in the control animals. In the animals treated with ACTH or cortisone, the secondary follicles were considerably diminished both in size and in number (Figs. 5 and 6). The number of mitotic figures present within the germinal follicles was markedly reduced. The medullary cords in hormone-treated animals (Figs. 7 and 8) were about as wide as those in the controls, but they stood out more conspicuously from the intermediate and central sinuses because of the great predominance of large transitional, preplasma, or plasma-cell precursor forms. These cells had a round-to-oval cell body and moderate to abundant basophilic, pyronine-positive cytoplasm, without a definite paranuclear vacuole. They had an eccentric, round nucleus which did not display the "cart-wheel" pattern of the adult plasma cell. In the control groups, the medullary cords were less conspicuous than they were in the animals treated with ACTH or cortisone because they contained a greater variety of cells which blended with the cell content of the sinusoids. Mitotic figures among the large transitional cells were seen occasionally in these latter areas in both control and hormone-treated groups. The peripheral sinuses of the animals treated with ACTH and cortisone contained fewer small lymphocytes and a larger proportion of transitional preplasma cell forms than did the controls. This difference was much more noticeable in the central sinusoids of the two groups, and was accentuated by the greater dilatation of the central sinuses in the hormone-treated groups. The number of eosinophils in the central sinusoids in the ACTH-treated rabbits was smaller than in the controls, and these cells were nearly absent in the cortisone-treated rabbits. In this group no differences were observed in comparing the fixed sinusoidal reticulum from the lymph nodes of the antigen-injected control rabbits with that from lymph nodes of the rabbits given ACTH or cortisone.

The methyl-green-pyronine stain revealed pyronine-positive cells scattered diffusely throughout the cortex in the lymph nodes of the hormone-treated animals; these scattered, large cells were not present in the lymph nodes of the control animals. There was a marked diminution of pyronine-positive material in the smaller secondary germinal follicles of the animals treated with ACTH or cortisone, in comparison to the controls; the intensity of the reaction was reduced, as well as the number of cells affected. In the medullary cords, however, the cytoplasm of the masses of large cells in the hormone-treated animals took an intense stain (Fig. 10). Similar cells were present also in the

medullary cords of the control animals, although they were mixed with cells of other types.

The popliteal lymph nodes on the uninjected sides of the animals showed more overlapping in weight values (Table II), as well as in histologic characteristics, than did those on the injected side. The width of the cortex and the size of the primary follicles were not different, but in the formation of the secondary follicles of lymphoblastic cells, the animals treated with ACTH or cortisone were comparatively deficient. With this failure in formation of the secondary reaction centers, there was also a comparative decrease in the number of mitotic figures, none of the hormone-treated animals displaying mitotic figures in the follicles. No significant differences were encountered in the medullary cords of these two groups. The peripheral and central sinuses were dilated less in the hormone-treated animals, and fewer small lymphocytes were present.

Group 3 Animals Killed Eight Days after Injection of Antigen

In group 3 there were no striking differences in the size of lymph nodes, in their histologic responses, nor in antibody production between the hormone-treated animals and the controls (Table III). In the injected foot pads, the organization of the necrotic exudate was much decreased in the cortisone-treated animals and slightly less so in the ACTH-treated animals, in comparison to the controls (Figs. 11 and 12). Along with the decreased evidences of fibroblastic and capillary proliferation, the formation of small lymphoid nodules, present in the controls, was not seen. In the small inflammatory foci that were present in the hormone-treated animals, mononuclear cells and transitional cell forms were seen rather than small lymphocytes.

The histologic response in the perinodal tissues did not differ significantly among the three groups. In all groups, the number of germinal follicles was increased, each usually having a thin rim of adult cortex surrounding the follicle; each of these follicles contained a prominent but small secondary follicle. In size, constituent cells, and mitotic activity, the lymph follicles of each group approximated one another. In the cortical sheets running from the peripheral sinuses to the medullary area, there was a tendency for the hormone-treated animals to have a decreased percentage of small lymphocytes and an increased percentage of large transitional cells. No obvious pyknosis of individual lymphocytes was seen in cortical nodal substance from either the control or the hormone-treated animals. A larger proportion of transitional cells in the peripheral and central sinusoids was noted in the animals treated with ACTH or cortisone; the total number of

TABLE III

Group 3: Animals Killed Eight Days after Injection of Antigen

Rabbits	Weight of adrenal gland		Serum titer, 4 days		Serum titer, 8 days		Right popliteal lymph node										Left popliteal lymph node									
							Weight		Width of cortex		Diameter of germinal follicles		Diameter of secondary follicles		Prominence of cortical transitional cells		Weight		Width of cortex		Diameter of germinal follicles		Diameter of secondary follicles		Prominence of cortical transitional cells	
	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range
	gm.	gm.					gm.	gm.	*	*	*	*	*	*	*	*	gm.	gm.	*	*	*	*	*	*	*	*
Control	.111	.092 to .132	1:123	1:20 to 1:280	1:325	1:20 to 1:640	.376	.173 to .689	1.7	1.0 to 2.5	1.2	1.0 to 1.5	0.6	0.3 to 1.0	1	± to 1	.130	.090 to .188	1.1	1 to 1.3	0.6	0.4 to 0.8	0.2	0 to 0.5	±	0 to ±
Received ACTH	.126	.091 to .143	1:106	1:80 to 1:160	1:345	1:160 to 1:640	.247	.205 to .385	1.7	1.2 to 2.0	1.1	1.0 to 1.2	0.8	0.4 to 1.1	1	± to 3	.148	.051 to .190	1.1	1 to 1.5	0.5	0.5 to 0.6	0.2	0 to 0.4	±	0 to 1
Received cortisone	.078	.060 to .120	1:140	1:80 to 1:160	1:260	1:80 to 1:480	.194	.150 to .219	1.3	1.2 to 1.6	0.8	0.7 to 1.0	0.5	0.4 to 0.6	2	1 to 3	.092	.065 to .132	0.9	0.6 to 1.0	0.5	0 to 0.8	0	0 to 0.2	±	0 to 1

* For quantitative values other than those in the metric system, see page 631.

cells in the sinusoids was much decreased as compared to the number in animals killed 2 or 4 days after injection of antigen. The medullary cords were narrower in the animals treated with ACTH or cortisone than in the controls. The sinusoidal reticulum had returned to normal in all groups.

Methyl-green-pyronine stains showed the same general pattern in all animals of the group, but the hormone-treated animals had a larger number of pyronine-positive cells in the cortex and medullary cords.

No significant differences were found in the histologic patterns of lymph nodes from the non-antigen injected side of hormone-treated and control animals.

Discussion

The significant deviations from the normal lymph node response induced by adrenocortical activity, observed in this experiment, were those of an inhibitory nature. This inhibition became apparent in the failure of the formation and persistence of the exuberant reaction centers in the antigenically stimulated lymph nodes which were seen in the control animals after 2 days. In rabbits treated with ACTH or cortisone, similar reaction centers have been reported to form within 6 hours after injection of the hormone.[2] It may have been that, in these experiments, this transitory state was not observed in the hormone-treated animals because,

after a period of 2 days, reconstitution of the germinal follicles in a lymph node not antigenically stimulated already would have been well established. The formation of these reaction centers must depend on local factors as well as on hormonal ones, since none was present in the nodes from the non-antigenically stimulated sides of the control animals (Table I), though small ones were found in similar nodes of the cortisone-treated animals, as a result of hormone effect.

Another inhibitory effect of ACTH and cortisone on the normal response of lymph nodes subjected to stimulation by foreign antigen was the suppression of the usual increases in the width of the cortex of the node. This may have resulted in part from the cytotoxic action of adrenal hormone and in part from the inhibition of mitotic activity and cellular growth. Apparently, the cytotoxic action affects primarily the small adult lymphocytes, since Dougherty and White[2] found no hormonal effect of a cytotoxic nature in the lymphoblasts and large lymphocytes. The inhibition of mitotic activity, a widespread biologic phenomenon, has been demonstrated in embryos, tumors, granulation tissue, and young growing animals.[38-43] This study presented evidence of both lymphocytic destruction in the cortex of the lymph nodes and mitotic inhibition in the cortex and secondary germinal centers of antigenically stimulated lymph nodes in animals treated with ACTH or cortisone. The greater uniformity of cell type seen in the cortex of lymph nodes in the hormone-treated animals of group 1 also may have represented an inhibition of the normal response, since many of the large cells present in the cortex of the lymph nodes from the control animals were actively proliferating lymphoblastic cells.

The suggested relationships of the pyronine-positive cytoplasmic materials to the ribose nucleic acids and to the formation of antibody protein have been presented by Ehrich and his co-workers[25] and by Harris and Harris.[14] Both of these groups of workers believed that the location of pyronine-staining material within the cells indicated a high ribose nucleic acid content. Ribose nucleic acid, in turn, is present in large quantities where active protein synthesis is occurring.[44] This material may be related to the production of intracellular protein for active cell growth, as well as to the synthesis of extracellular proteins.[45] The formation of ribose nucleic acid within the cytoplasm of cells of the medullary cords was not inhibited by ACTH or cortisone. The observed decrease in the presence of pyronine-positive cells within the secondary follicles may well be the result of a failure of supply of cells, due to inhibition of mitotic activity. The abundant formation of pyronine-positive material within the cells of the medullary cords, where it is usually in greatest concentration, and also throughout the

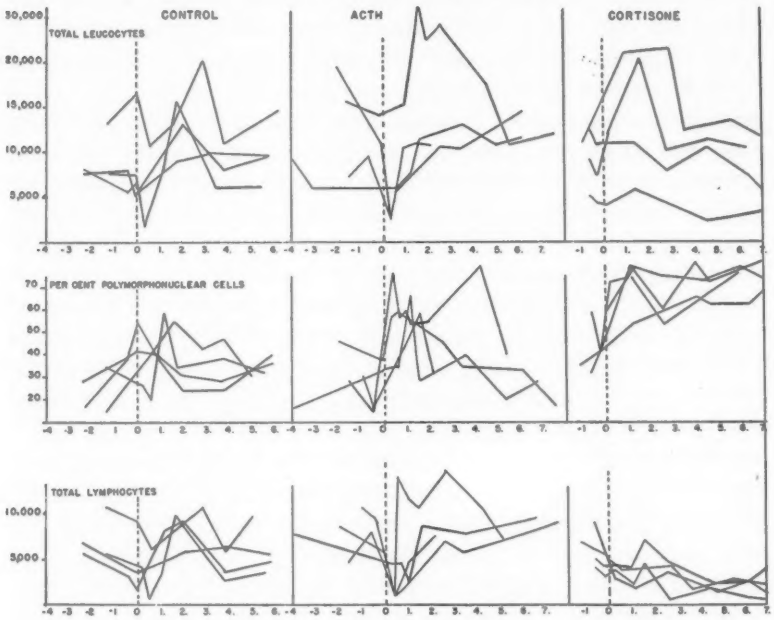
cortex, suggests that the suppression of antibody formation noted by others is related more to the mitotic inhibitory action of adrenocortical hormone than to any failure of the individual cells to produce antibody —that is, it is due more to a total reduction in the lymphoid tissue mass at peak antibody response than to any alteration in cell function during this response.

According to Ringertz and Adamson,[26] the actively dividing cells of the secondary germinal follicles may develop into either plasma cells or lymphocytes. They distinguished the intermediate preplasma cell forms from other immature cells of the lymph node by the presence of pyronine-positive material in their cytoplasm and nucleus. The presence of pyronine-positive material in the cells of the germinal follicle suggests that they may have a prominent rôle early in the course of antibody production. Ehrich[46] believed that these foci of extreme mitotic activity in the cortex were not the same as the later developing "Fleming's centers," and called them pseudo-secondary nodules, but did not explain their relation to antibody formation.

Bjørneboe and Gormsen,[24] using repeated injections of a variety of antigens, found that the plasma cells, which they believed to be the source of antibody, first appeared in considerable numbers about the ninth or tenth day after the start of injections; moreover, they found that these cells appeared in areas away from the follicles. Fagraeus[47] found the splenic red pulp to be a more active source of antibody than the follicles, and this relationship coincided with the position of the plasma cells in the pulp. In the present experiment, few adult plasma cells could be identified, yet significant antibody titers developed. Considerable numbers of pyronine-positive cells were present in the large secondary germinal follicles in the control animals. These facts indicate that there is a difference in the distribution of pyronine-positive material in the lymph nodes in response to a single, short-term antigenic stimulus, in contrast to a chronic, multiple antigenic stimulation in which a hyperimmune state develops. In contrast to the observations reported by Harris and Harris,[14] in the present study, no pyronine-positive material was found in the small mature lymphocytes in any of the groups.

In these experiments, the failure of either ACTH or cortisone to affect significantly the antibody titer is disturbing. Most of the experiments used in assessing the action of ACTH or cortisone have utilized repeated injections of antigen extending over a period of several weeks. Thus, they are concerned more with a hyperimmune than an immune reaction. In the ACTH-treated animals, several other factors also may be considered in relation to the failure of antibody suppression. First,

a local antagonism to ACTH[48] may have developed, since the hormone was injected in a fairly circumscribed area of the left thigh. Second, a large dose of ACTH may have given rise to an exhausted state in the adrenal gland. Third, ACTH in rabbits, as in man,[7] may be able to effect only a temporary depletion of lymphocytes. In keeping with this suggestion, in this series a sharp initial drop in the absolute lymphocyte count was noted (Text-fig. 1) in the peripheral blood, both in control



Text-figure 1. Serial leukocyte levels of the peripheral blood from the rabbits of group 3. The point "O" days represents the time of injection of antigen.

and in ACTH-treated groups. A subsequent rise to a control level occurred in both these groups, but the lymphocyte counts in the ACTH-treated animals lagged slightly in their rise. In contrast to this, the lymphocyte counts of the cortisone-treated animals became lower as the experimental period lengthened. The failure of ACTH and cortisone to inhibit uniformly or completely the formation of antibody has been noted by nearly all other workers.[28-31,33] Germuth and his coworkers,[30] however, by administering cortisone from the onset of immunization, were able to inhibit antibody formation almost completely.

The histologic differences between the lymph nodes of the hormone-treated and control groups were always far less conspicuous in the

popliteal nodes of the side not receiving injected antigen. Thus, an antigenic stimulus accentuates in lymph nodes the histologic differences arising from the influence of adrenocortical substances. Some of these changes are exaggerations of those seen in nodes not so stimulated. These include the pyknosis and loss of small lymphocytes, swelling of the sinusoidal reticulum cells, phagocytosis of cell elements, and arrest of mitotic figures in metaphase. Other effects noted in these experiments are not usually found in lymph nodes not stimulated antigenically. These include suppression of reaction centers, of increases in width of the cortex, of the formation of germinal follicles, and of mitosis within the germinal follicles. A greater uniformity of cell content of the peripheral sinuses and medullary cell cords was present in the hormonally influenced lymph nodes. Probably this was a consequence of the lysis and inhibition of formation of small lymphocytes.

Conclusion

Rabbits receiving adrenocorticotropic hormone or cortisone during the period of maximal antibody production caused by a single antigenic stimulus showed a significant depression of the normal histologic response in the affected regional lymph nodes. This repressive influence was found in the reduced mass of the affected lymph nodes, in their narrowed cortex, in their lack of reaction centers, and in the partial inhibition of the formation of germinal centers and mitotic figures. The adrenocortical activity did not inhibit the formation of transitional or preplasma cell forms.

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DESCRIPTION OF PLATES

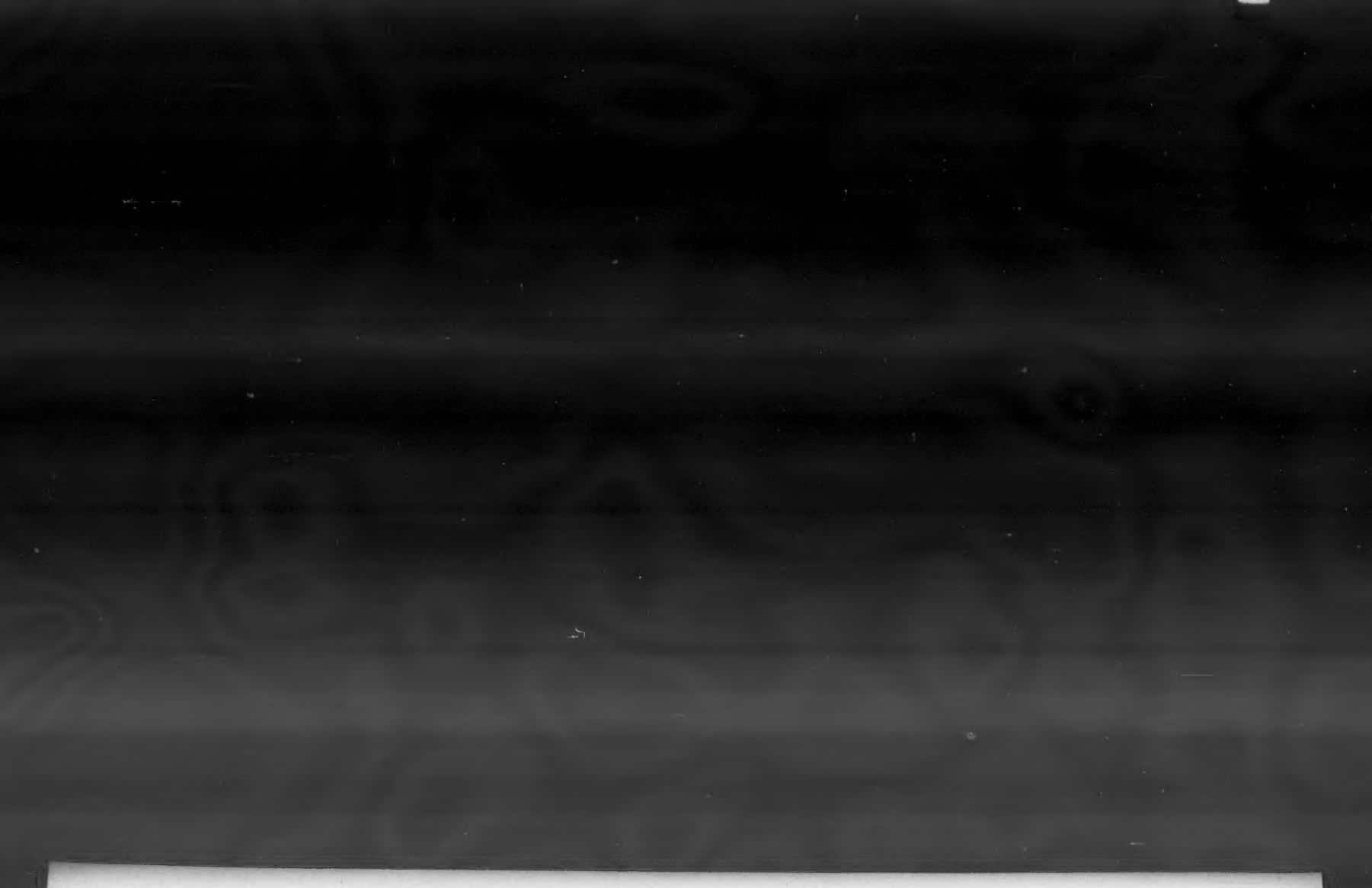
PLATE 88

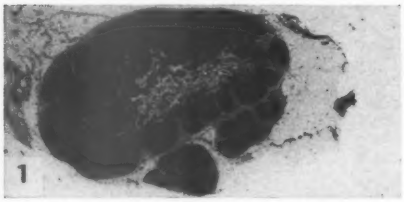
FIG. 1. Right popliteal lymph node from control animal 48 hours after injection of antigen. The cortex is wide and densely cellular. Toluidine blue and eosin stain. × 4.

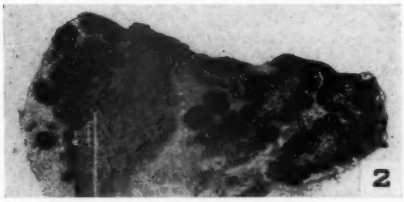
FIG. 2. Right popliteal lymph node from ACTH-treated animal 48 hours after injection of antigen. The cortex is narrow and does not encroach on the medullary cords. Toluidine blue and eosin stain. × 4.

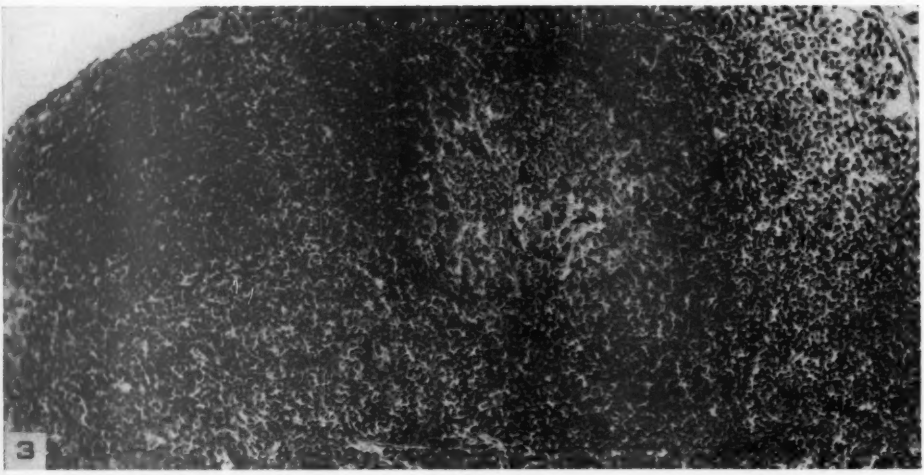
FIG. 3. Right popliteal lymph node from control animal 48 hours after injection of antigen. The cortex is broad and cellular and contains a large reaction center. Toluidine blue and eosin stain. × 125.

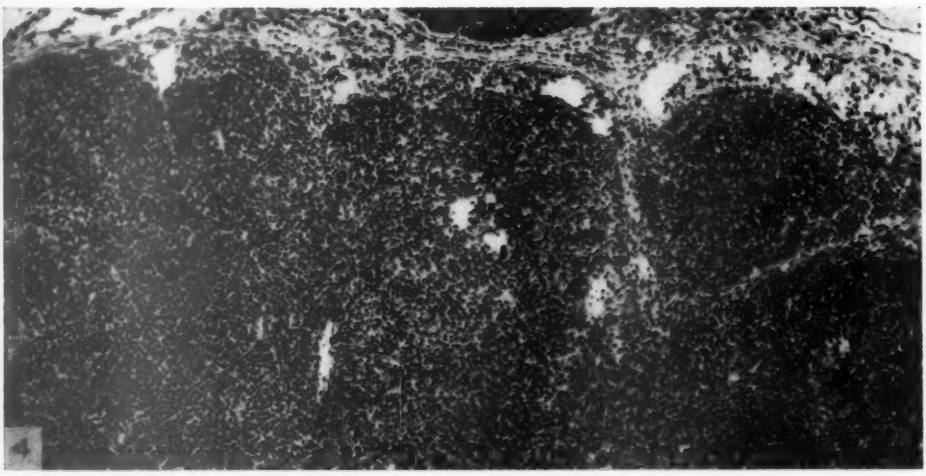
FIG. 4. Right popliteal lymph node from ACTH-treated animal 48 hours after injection of antigen. The perinodal tissue is infiltrated. A large thrombus is present in a perinodal lymphatic. No well outlined follicles are present. Toluidine blue and eosin stain. × 125.









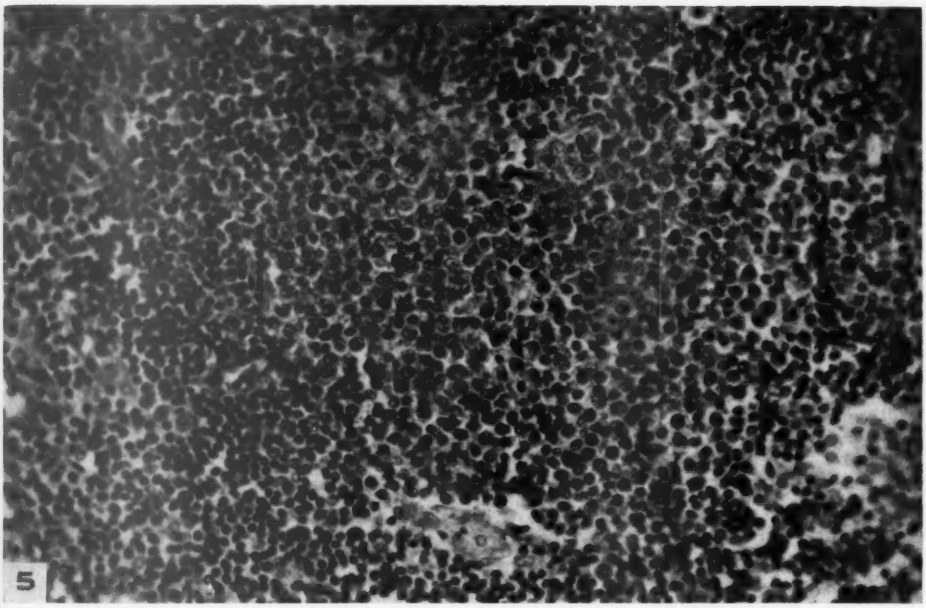


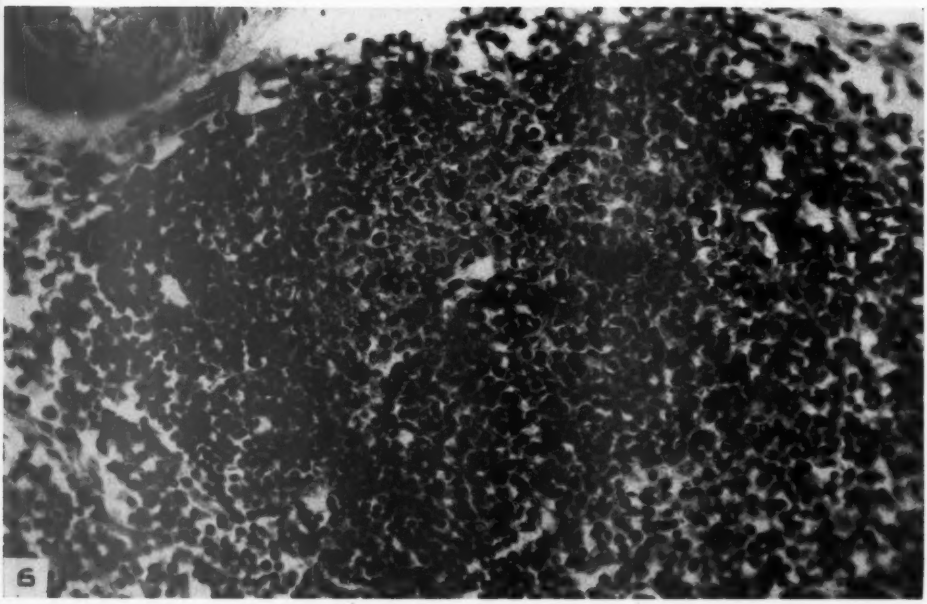
Craig Lymph Nodes after ACTH or Cortisone

PLATE 89

FIG. 5. Right popliteal lymph node from control animal 4 days after injection of antigen. A large germinal follicle with prominent secondary center containing many mitotic figures is present. Many small lymphocytes surround the secondary follicle. Toluidine blue and eosin stain. × 300.

FIG. 6. Right popliteal lymph node from ACTH-treated animal 4 days after injection of antigen. The germinal follicle is small, with minimal mitotic activity. Few small lymphocytes are seen in the perifollicular area. Toluidine blue and eosin stain. × 300.





Craig Lymph Nodes after ACTH or Cortisone

PLATE 90

FIG. 7. Right popliteal lymph node from control animal 4 days after injection of antigen. The medullary cords contain a variety of small lymphocytes, reticulum cells, and transitional cells. Toluidine blue and eosin stain. × 300.

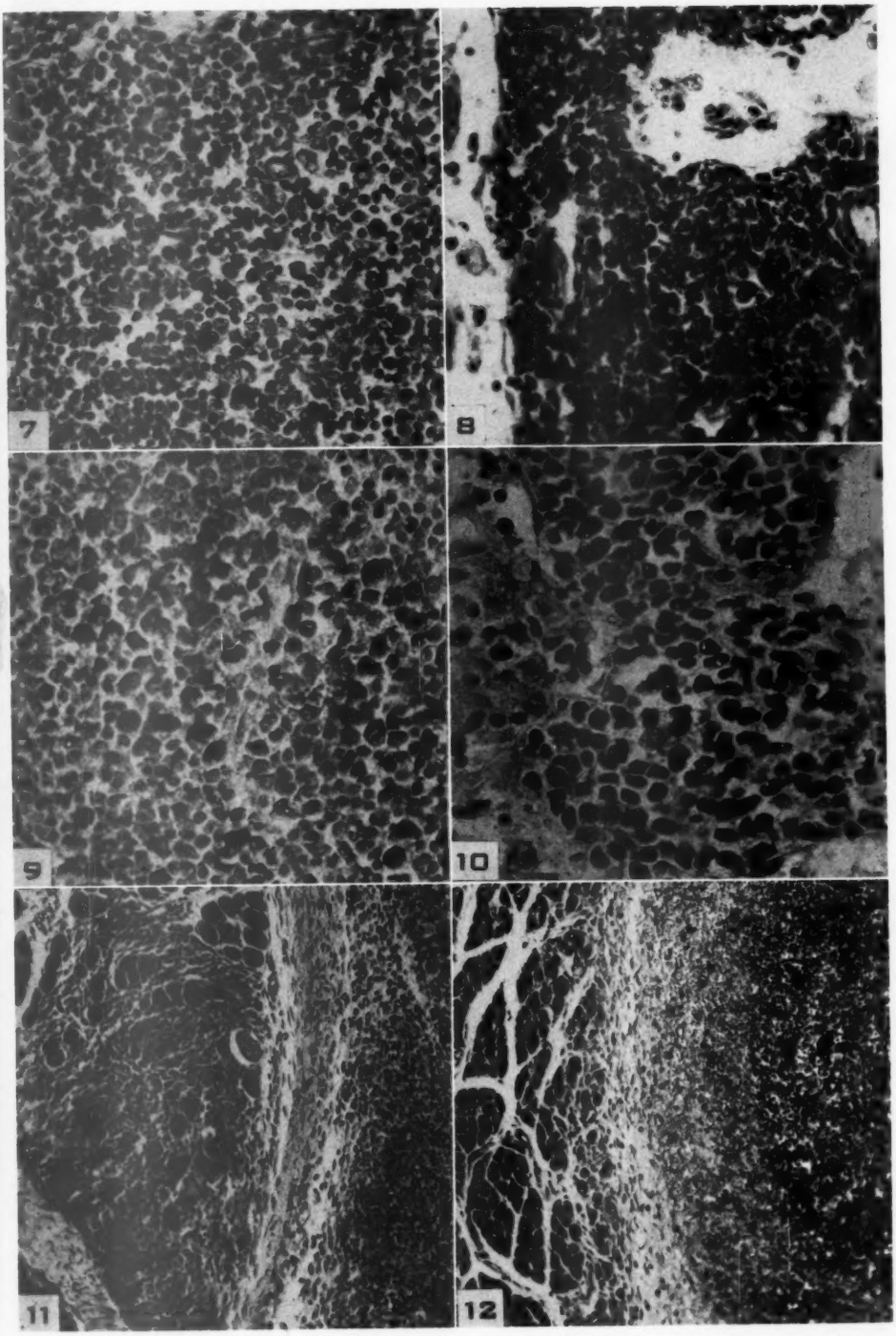
FIG. 8. Right popliteal lymph node from ACTH-treated animal 4 days after injection of antigen. The medullary cords consist predominantly of transitional preplasma cells. Toluidine blue and eosin stain. × 300.

FIG. 9. Right popliteal lymph node from control animal 4 days after injection of antigen. The germinal follicle contains many pyronine-positive cells with dark cytoplasm. Methyl-green-pyronine stain. × 400.

FIG. 10. Right popliteal node from ACTH-treated animal 4 days after injection of antigen. The medullary cord transitional cells stain intensely with pyronine, as do a few transitional cells in the sinusoids. The reticulo-endothelial cells of the sinusoids stain better. Methyl-green-pyronine stain. × 400.

FIG. 11. Right foot pad from control animal 8 days after injection of antigen. The interstitial exudate is well organized and replaced by sheets of small lymphocytes. Toluidine blue and eosin stain. × 125.

FIG. 12. Right foot pad from cortisone-treated animal 8 days after injection of antigen. The interstitial exudate remains without organization, and few lymphocytes are present. Toluidine blue and eosin stain. × 125.



Craig Lymph Nodes after ACTH or Cortisone

HEMANGIOPERICYTOMA: HISTOLOGIC AND TISSUE CULTURE STUDIES *

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In 1942 Stout and Murray[1] studied nine tumors which they called hemangiopericytoma. These were vascular neoplasms presenting a histologic structure different from the glomus tumor, vascular fibrosarcoma, hemangioendothelioma, and paraganglioma. Although the histologic appearance of the hemangiopericytoma may vary, certain features are diagnostic. This neoplasm contains numerous capillaries which often are indistinct in routine sections stained by hematoxylin and eosin. These vascular channels are surrounded by a connective tissue sheet of varying thickness. About this cuff of connective tissue are the tumor cells which, for the most part, are round to ovoid with moderately vesicular nuclei. The tumor lacks the organoid pattern of the glomus tumor. Reticulum stain demonstrates the vascular channels as well as the extraluminal position of the tumor cells. Delicate reticulum fibers may encircle individual cells although in some of these tumors this tendency has not been as noticeable as in glomus tumors. The origin of the tumor cell is believed to be the capillary pericyte described by Zimmermann.[2] This cell is related to smooth muscle cells but lacks myofibrils. It is probably identical with the "epithelioid" cell of the glomus tumor. The morphologic variations demonstrated by the pericyte perhaps explain the inconstant appearance of the tumor cells in the hemangiopericytoma. Tissue from one of these neoplasms has been explanted *in vitro* by Murray.[3] Since its growth was not characteristic enough to warrant the exact identification of the tumor cell type, the cell of origin has remained hypothetic. However, Murray and Stout[4] have grown characteristic cells from glomus tumors and they believe these cells to be identical with the pericytes of Zimmermann.

Recently we have had the opportunity to explant tissue from a hemangiopericytoma as well as to perform special histologic studies.

REPORT OF CASE

The patient was a white woman, 27 years old, who had a recurrent tumor in the soft tissue of the posterior aspect of the right thigh. Five years previously she had

* This work was supported, in part, by a grant from the Damon Runyon Memorial Fund for Cancer Research.
Received for publication, January 4, 1952.
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experienced low back pain which occasionally radiated down both thighs, and at that time examination revealed a slight prominence of the right gluteal fold but no mass could be palpated in this region. Two years previously she observed a moderately tender mass in the posterior aspect of the thigh which was locally excised. Its location was distal to the gluteal fold and had reached the size of "a pound of butter" in the period of 2 weeks prior to excision. One and a half years following this operation the mass reappeared in the same location and was again excised. It was described as being the size of a "goose egg." Within 6 months following the second operation the mass was again noted by the patient at the site of previous excision. She was then referred to the Cleveland Clinic for investigation.

On examination there was a non-tender, firm, irregular, slightly fixed mass behind the medial to the upper one third of the right femur in the subcutaneous tissue below the abductor tendons. The surrounding tissue was indurated and tender. The remainder of the examination was negative. Roentgenograms revealed an irregularly rounded density lying in the soft tissue of the thigh medially and posteriorly. It measured 5.5 by 6 cm. The bones of the leg were of normal appearance. Roentgenogram of the chest was negative.

The tumor lay over the hamstring muscles, was not encapsulated, and was excised with a large portion of the semitendinosus muscle which it had invaded. The wound healed without complication and the patient was discharged 9 days after operation. Six months later the patient was found to be in good general health without any evidence of tumor.

Pathologic Studies

Gross Findings. The specimen consisted of an elliptical portion of skin, measuring 15 by 1 cm., and underlying soft tissue, including a large segment of skeletal muscle, measuring 15 by 9 by 6 cm. (Fig. 1). Within the soft tissue was an irregularly shaped, rather circumscribed but not encapsulated mass, measuring approximately 10 by 8 by 5 cm. The cut surface was bosselated, soft, pinkish-tan, and homogeneous, with irregular bands of dense white tissue of varying thickness coursing throughout its substance. The neoplastic tissue had invaded the muscle but not the overlying skin and did not extend to the lines of excision.

Microscopic Findings. Sections of tissue fixed in Zenker's solution and stained with hematoxylin and eosin and methylene blue revealed a neoplasm formed by aggregates of spindle shaped to round cells (Figs. 2, 3, 4, and 5), for the most part having definite, pale-staining cytoplasm. The nuclei were also round to ovoid, vesicular, and uniform. Mitotic figures were rather frequent but atypical forms were not evident. Scattered throughout the cell aggregates were endothelium-lined spaces, some of which were not distinct. In some areas tumor cells appeared to be radiating about these small vessels but without an organoid arrangement. Connective tissue septa of varying thickness separated cell masses. Larger endothelium-lined spaces in these trabeculae contained tumor masses within their lumina. The neoplasm was not encapsulated in the sections studied. However, it appeared to be growing *en masse*, pushing aside surrounding skeletal muscle.

Various special stains were used to study some of the properties of this tumor. Wilder's reticulum stain outlined the vessel membranes and demonstrated the extraluminal position of the tumor cells. The reticulum was delicate and surrounded cell groups as well as a few individual cells. Cellular fibrils were not seen in sections stained with phosphotungstic acid hematoxylin. No intracellular fat could be demonstrated with Sudan IV and no glycogen was seen when sections were stained with Best's carmine. The acid phosphatase reaction was negative. Incubation of fresh neoplastic tissue with neutral red failed to reveal any granules within tumor cells.

In Vitro Studies

Material and Methods. The portion of tumor used for explants was chopped into 1 to 2 mm. fragments. Twelve fragments were planted using Maximow's plasma clot, hanging drop method, and 8 were cultured in roller tubes.

In the plasma hanging drop method the constituents of the medium were: one drop of human serum, one drop of chicken plasma, and one drop of 25 per cent embryo extract containing 50 mg. per cent $NaHCO_3$ and 0.003 per cent phenol red. The balanced salt solution (Hanks) was supplied by using it to reconstitute the chicken plasma. The cultures were washed and one drop of a mixture of fresh embryo extract and serum was added after 3, 7, 11, and 15 days of culture. In the roller tube method the clot was formed by mixing one drop of 25 per cent embryo extract with one drop of chicken plasma. The liquid supernatant was composed of 10 per cent embryo extract, 40 per cent human serum, and 50 per cent Hanks' solution containing 50 mg. per cent $NaHCO_3$ and 0.003 per cent phenol red. The supernatant liquid was replaced by fresh medium at 2-week intervals. In both methods an initial pH of 7.6 was attained by gassing with 21 per cent oxygen, 8 per cent carbon dioxide, and 71 per cent nitrogen.

Results. In the plasma hanging drop cultures a few fibroblasts as well as endothelial cells grew from the edge of the explant after 36 to 48 hours. The endothelial cells appeared first as spikes (Fig. 6) and then as hollow tubes. The fibroblasts appeared singly or sparsely scattered among round cells. They were of the typical elongated, spindle-shaped variety.

The numerous "round cells" which began appearing from the second to the seventh day were a striking feature of the growth. This cell type is depicted in Figures 7 and 8. The identity of these cells as a major portion of the original cell population was demonstrated by their similarity to the cells seen in thin, transparent areas in the main body

of the explants. The cells wandered out singly but they were often observed in small clumps which were sometimes adjacent to the capillary tufts. No giant cell forms were evident. The diameter of the "round cells" varied from approximately 15 to 20 μ. The cytoplasmic membrane of most of these cells could be seen to move although the roundness of the cells did not change. The cytoplasm was granular. This granularity increased with age and was not noticeably altered by washing. Fat granules were not noticed even in old cultures of 20 or 25 days. In unfixed preparations the nuclei which were difficult to discern appeared round to oval, measuring approximately 7 to 10 μ in diameter. When nucleoli could be seen, only one per cell was present. In fixed and stained preparations, however, the nuclei were distinct. There was a marked similarity between these cells and those studied in the paraffin sections.

In addition, another cell type was identified in preparations fixed and stained after 10 days (Figs. 9 and 10). These cells were smaller than those previously described and were scattered beyond the main body of the explants, intermingled with small numbers of fibroblasts. They were discretely distributed. The cytoplasm was scant but contained extended filamentous processes which grew in three dimensions and thus were difficult to demonstrate in photographs. The cell nuclei were hyperchromatic, globular, and contained one or two nucleoli when these structures were present. In some respects these cells resembled the pericytes grown by Murray and Stout[4] from an infiltrating glomus tumor.

The roller tube cultures were characterized by the dense growth of the "round cells" described in the hanging drop method. A greater tendency for cell clumping was noted in growths by this method, especially in cultures 40 to 45 days old.

In general, the tumor grew rather rapidly during the first 10 days. The pH dropped to 6.8 and 7 after 48-hour intervals. The rate of pH drop slowed after several weeks, at which time the buffer was able to maintain pH 7.4 quite constantly.

Discussion

The results of the explant studies offer evidence as to the cell type encountered in hemangiopericytoma. Cells resembling those described by Murray and Stout[4] as pericytes grown from an infiltrating glomus tumor were demonstrated. They were scattered singly beyond the main body of the explant. Their nuclei were globular and contained one or two nucleoli. However, the cell processes observed were coarser and it was more difficult to demonstrate the extensive branching de-

scribed by these authors. This may be due to the fact that our preparations were stained with iron hematoxylin only and not by the intense silver impregnation methods. Murray and Stout stated that these ramose processes are often difficult to demonstrate by ordinary staining methods. In addition actual encrustation of capillaries by these cells was not present. Further, the composition of the culture media employed in this study differed from that used by Murray and Stout and this could explain differences in the behavior and appearance of the cells.

The small "round cells" which grew abundantly near the main explant resembled the original tumor of the explant and showed a tendency to cluster about capillary tufts. Because of the slight granularity of their cytoplasm, motility, and movement of their cytoplasmic membrane the possibility that these cells represented macrophages had to be considered. However, no active phagocytosis or movement by pseudopods was observed. The contour of the cytoplasm was consistently round and, as previously mentioned, cells of this type constituted a major portion of the tumor material, histologic sections of which failed to reveal macrophages. Further, the cells from fresh neoplastic tissue failed to be supravitally stained with neutral red. This cell may well represent a morphologic variant of the pericyte.

Histologic sections of this neoplasm demonstrated numerous mitotic figures, more than are usually encountered in hemangiopericytoma. Also the presence of tumor masses within endothelium-lined spaces supports a diagnosis of malignant hemangiopericytoma. Although the patient has been followed for 6 months without recurrence, prognosis must be guarded since the case closely resembles that described recently by Forrester and Houston[5] in which widespread metastases resulted in death 5 years after removal of the primary growth.

Summary

Special histologic and tissue culture studies were applied to a hemangiopericytoma of the thigh. The neoplasm was considered malignant because of frequent mitotic figures and vascular invasion.

In tissue culture studies, in addition to fibroblasts and endothelial cells, cells of two other types were demonstrated. Those of one type resembled the cells grown from a glomus tumor by Murray and Stout.[4] These authors described these cells as pericytes. The other cells were larger and round. It is conceivable that these represent a morphologic variant of the first type described.

These studies present *in vitro* characteristics as well as other special histologic features of the hemangiopericytoma.

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DESCRIPTION OF PLATES

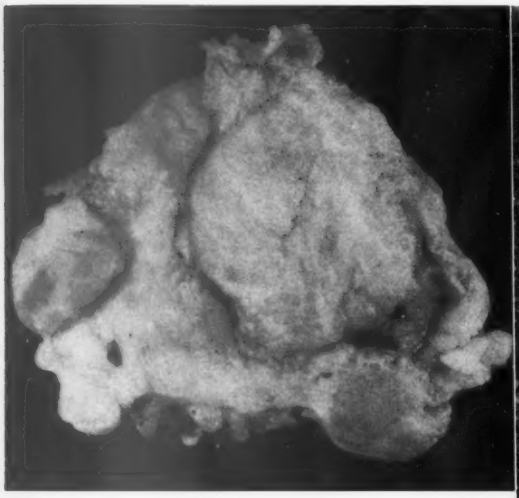
PLATE 91

FIG. 1. Cut surface of tumor.

FIG. 2. Photomicrograph demonstrating perithelial arrangement of spindle and round cells. Hematoxylin and eosin stain. × 115.

FIG. 3. Photomicrograph demonstrating frequent mitotic figures. Hematoxylin and eosin stain. × 230.

FIG. 4. Neoplasm in endothelium-lined space. Hematoxylin and eosin stain. × 65.



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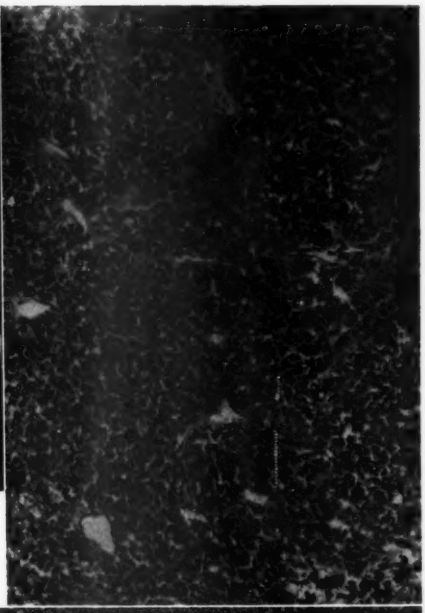

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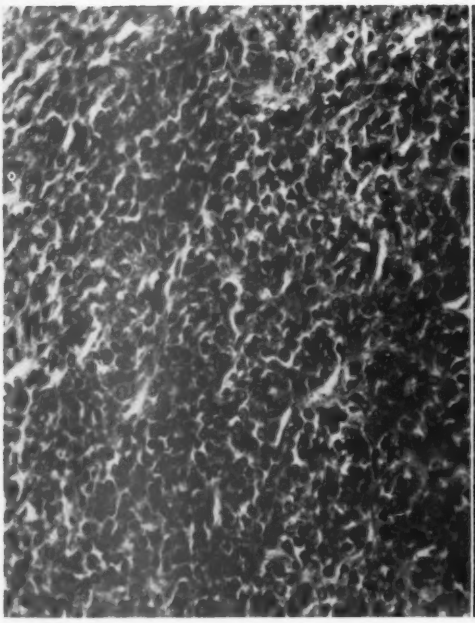

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Fisher, Kaufman, and Mason Hemangiopericytoma

PLATE 92

FIG. 5. Section stained by Wilder's reticulum method demonstrating extraluminal position of tumor cells and distribution of reticulum. × 70.

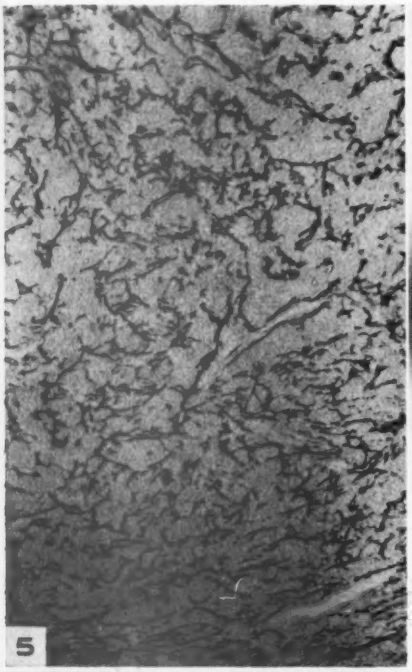
FIG. 6. Explant after 24 hours. Collected extensions of cells represent endothelial growth, unfixed. × 100.

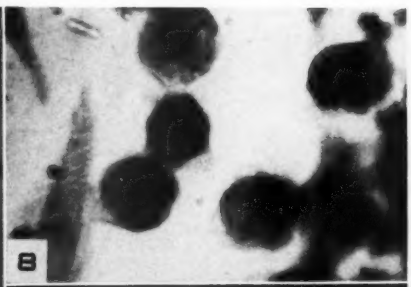
FIG. 7. Explant after 7 days. "Round cells" scattered among fibroblasts and also around endothelial sprouts, unfixed. × 180.

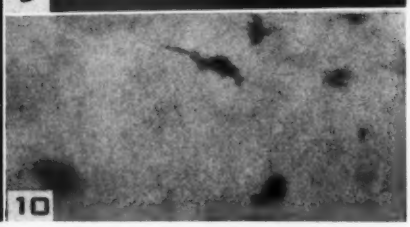
FIG. 8. "Round cells" and fibroblasts. Five "round cells" are present in the field and parts of two fibroblasts are found at the left, unfixed. × 980.

FIG. 9. Explant after 10 days. Cells with bulging globular nuclei, scant cytoplasm, and filamentous processes. Formalin vapor fixation. Iron hematoxylin stain. × 490.

FIG. 10. Explant after 10 days. Cells with bulging globular nuclei, scant cytoplasm, and filamentous processes. Formalin vapor fixation. Iron hematoxylin stain. × 490.







HAMARTOMA OF THE SPLEEN

A Report of Four Cases *

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Benign splenic tumors composed of abnormal mixtures of normal splenic elements have been reported infrequently for over 100 years. They have been given various names, including splenadenoma, fibrosplenoma, nodular hyperplasia of the spleen, lymphoma of the spleen, splenoma and, more recently, hamartoma of the spleen.

Only 23 cases are sufficiently well documented for comparison. Descriptions of the older tumors have been summarized by Mordasini,[1] who added 6 cases of his own. Later reports by Fischer,[2] Sweet and Warren,[3] Kirkland and McDonald,[4] and Fasanotti[5] gave 5 additional examples. All were benign and most were spherical, sharply demarcated, firm nodules varying from 0.8 to 23 cm. in diameter, appearing dark red to pinkish gray. They were solitary tumors in all but 3 cases. Microscopically they consisted of various normal splenic elements in abnormal quantitative or structural relationships, which, in the main, could be separated into two forms, the lymphoid and the pulposal, depending upon whether white or red pulp predominated within the tumor.

Recently we observed four similar splenic nodules, the first in a surgical specimen and 3 in autopsy specimens.

Case 1

R. B., a white female, 60 years old, was brought to the hospital following a blow to the left upper portion of the abdomen. She was nauseated, vomiting, in shock, and had tenderness to deep palpation in the epigastrium. Her past medical history was not helpful. The hemoglobin was 15 gm. per cent on admission, but fell within a few hours to 11 gm. per cent. The white blood cell count was 10,800 per cmm. with a normal differential count. Urinalysis and chemical examinations of the blood gave results within normal range.

An exploratory laparotomy revealed a ruptured spleen. This was removed and the patient left the hospital 2 weeks later completely recovered.

The spleen weighed approximately 300 gm. In the midportion the capsule was ruptured and one end was covered by clotted blood. This hemorrhagic area contained a large spherical tumor 6 cm. in diameter, sharply demarcated from the surrounding splenic tissue. The tumor was soft, grayish white, and contained punctate areas of hemorrhage through the central portion. The line of rupture in the spleen lay im-

* Received for publication, January 4, 1952.

mediately adjacent to this nodule and partially dissected the tumor from the pulp. The remaining parenchyma appeared normal.

Microscopically, the tumor had a relatively uniform, lymphoid appearance. The great majority of cells were small lymphocytes. Small spindle-shaped cells that appeared to be fibrocytes or small reticulum cells were scattered diffusely among the lymphocytes. Occasional large reticulum cells were present. Many sinusoids, both large and small, coursed irregularly through the tumor and for the most part were devoid of cells. Erythrocytes were found in small foci scattered through the tissue. Special stains demonstrated a background composed of a loose network of fine collagen fibers. No follicles or trabeculae were present. The edge of the tumor was distinct but not encapsulated. The adjacent tissue was distorted by pressure from the tumor, the sinusoids and trabeculae tending to form concentric layers about the mass and the follicles flattened and closer together than in the remainder of the spleen. At a distance from the tumor there were no abnormal histologic features.

Case 2

W. M., a white male, 68 years of age, entered the hospital because of recurrent symptoms of congestive heart failure resulting from hypertensive cardiovascular disease. His response to therapy was poor and he gradually deteriorated until death. Laboratory studies, including hematologic examinations, were within normal limits during his numerous hospital admissions.

The principal findings at necropsy were marked cardiac hypertrophy, marked coronary arteriosclerosis, and evidence of severe chronic passive congestion in all viscera.

The spleen was enlarged and irregularly mottled due to arterial embalming. There were irregular areas of hyaline perisplenitis. The parenchyma was soft and for the most part dark red, showing poor fixation. A roughly spherical, sharply circumscribed nodule, 2 cm. in greatest diameter, was found in the central portion of the spleen near the hilus. This was of the same consistency and color as the surrounding splenic tissue.

Microscopically, the tumor was hemorrhagic and contained many areas of degeneration which were most marked in the central portion. Most of the tumor was so engorged with erythrocytes that the pattern was obscured, but where cellular detail was good the appearance was similar to red pulp. Sinusoids distended by erythrocytes were separated by a loose stroma which contained many cellular elements among which lymphocytes and plasma cells were common. There were many reticulum cells and monocytes or small macrophages. Scattered neutrophils were noted and small foci of immature granulocytes were scat-

tered irregularly through all areas of the tumor. Megakaryocytes were prominent. No erythrogenesis could be distinguished. No trabeculae or lymphoid follicles were present and arteries were infrequent and poorly developed. Azo-carmine stains revealed a very loose, irregular, extremely fine network of collagen fibers. The surrounding splenic tissue was compressed and the follicles immediately adjacent to the tumor were distorted into elongated bands. The trabeculae adjacent were bowed-out and formed a pseudo-capsule along one edge of the tumor. More distally the spleen appeared normal except for congestion. There were no megakaryocytes and no evidences of myeloid metaplasia in the surrounding spleen.

Case 3

S. S., a white male, 79 years old, entered the hospital with congestive heart failure of several years' duration. He did not improve with therapy and expired quietly 10 days after admission. Hematologic examinations during his many admissions were always normal.

The principal necropsy findings were cardiac hypertrophy with myocardial fibrosis and severe calcific coronary arteriosclerosis. All organs showed changes due to severe chronic passive congestion.

The spleen weighed 600 gm. The capsule was tense and bluish purple. The pulp was a relatively homogeneous dark red with indistinct follicles and trabeculae. A small, sharply circumscribed, spherical nodule, 9 mm. in diameter, was present 2 cm. under the capsule in the midportion of the spleen. This was light pink, firm, and slightly elevated above the surrounding splenic tissue when sectioned.

Microscopically, the tumor was composed of a loose, fibrous tissue through which many large sinusoids coursed irregularly. These sinusoids were lined by closely situated endothelial cells whose nuclei protruded prominently into the lumina. The sinusoids were devoid of all but occasional granulocytes and mononuclear cells. The fibrous stroma contained a few small aggregates of lymphocytes and plasma cells as well as a light, diffuse infiltration of leukocytes and mononuclear cells. A few small arteries with thin muscular walls were found within the tumor along with a single trabecula which extended in from the surrounding splenic tissue. No evidence of any capsule or lymphoid follicles was found within the tumor.

The adjacent parenchyma showed compression by the tumor although this was not as marked as in the 2 cases previously described and was not apparent in the entire circumference of the tumor. The follicles were small with inconspicuous germinal centers. The trabeculae were more fibrotic than normal but contained many erythro-

cytes, histiocytes, and varied leukocytes. A localized area of acute splenitis with heavy infiltrations of neutrophils was present a short distance from the tumor. No other areas of acute inflammation were noted in other sections. The penicilliary and trabecular arteries were very sclerotic.

*Case 4**

J. S. was a white male, 39 years of age, who entered the hospital after 2 weeks of constant diarrhea, nausea, and vomiting. The stools were watery and blood tinged, and on admission the patient was severely dehydrated with the blood pressure at shock levels. Laboratory studies were all within normal range except for a moderate leukocytosis. He failed to respond to therapy and expired.

The principal necropsy findings were diffuse polyposis of the rectum with ulceration, dehydration, and left pleural effusion.

The spleen weighed 200 gm. The capsule was smooth. On section the pulp was dark red and firm. A sharply circumscribed nodule, 2 cm. in diameter and light gray, was found within the substance of the spleen.

Microscopically, the tumor tended to fade into the surrounding normal parenchyma, being better seen under low power than high. In a few areas there was evidence of pressure effect upon the surrounding tissue, with the sinusoids and trabeculae tending to form concentric layers along the edge of the mass. Within the tumor were all elements of normal splenic tissue, but trabeculae and follicles were small and widely scattered. The sinusoids were similar to those in the adjoining normal spleen, being easily visualized in both locations because of their cell-free lumina. The trabeculae within the tumor were thicker, denser, more fibrotic, and contained only a few erythrocytes in contrast to the surrounding pulp. The free cells consisted primarily of lymphocytes with occasional neutrophils, plasma cells, and histiocytes. No evidence of myeloid metaplasia was noted. The surrounding spleen revealed no histologic abnormalities.

Comment

Various ideas as to the etiology and histogenesis of these tumors have been advanced. Among these may be mentioned areas of organizing hemorrhages, degenerating angiomas, hyperplasia secondary to splenic inflammation, and accessory spleens within the spleen. After extensive examination of these theories, Mordasini[1] believed they were all incorrect and that the lesion is best explained as a hamartoma—a term proposed by Albrecht for congenital tumor-like errors in development. Most subsequent writers concur. However, no convincing evidence of the congenital origin of the nodules has as yet been advanced.

* Courtesy of Dr. Kano Ikeda.

The few cases reported would indicate that these tumors are extremely uncommon. While the larger ones such as that described in case 1 are undoubtedly rare, similar smaller tumors could very easily be overlooked in routine necropsies.

The ages at which the tumors were identified varied from 5 to 84 years in the 26 cases reviewed and, since most were incidental postmortem findings, they were found more frequently in the older age groups. Distribution between the sexes was equal and none was associated *per se* with any abnormal laboratory or clinical findings other than those due to size, rupture, or pressure on the surrounding viscera.

Microscopically, they presented a variety of appearances depending upon which of the normal splenic elements predominated and the 4 cases presented are representative of the varying morphologic patterns previously described. The uniting factor in all is their composition of normal splenic elements in abnormal quantitative or structural relationship. Lubarsch[6] and Mordasini[1] both divided such tumors into two main classes, the lymphoid and the pulposal, in which, respectively, either white pulp or red pulp is more prominent. Case 1 of our group would thus be classified with the lymphoid variety and cases 2, 3, and 4 with the pulposal despite rather wide variation in the histologic structure of the last 3 cases. Since apparently almost any mixture can occur, rigid classification does not seem possible.

None of the tumors possessed capsules. Pseudo-capsules from compressed trabeculae were noted and the regular splenic capsule partially surrounded some of the larger growths originating in the subcapsular region. This partial envelopment by the regular splenic capsule is the feature referred to in Kirkland and McDonald's[4] summary of their cases rather than an independent capsule about the tumor.

The lymphoid variety may resemble malignant lymphoma grossly and to a certain extent microscopically. It is important, therefore, that their identity is not mistaken and an incorrect prognosis given in the event of surgical removal. The presence of numerous sinusoids in these benign tumors will be of aid in the differentiation of most cases.

Summary

This study was based on four examples of a benign splenic tumor. This is interpreted as due to congenital errors in development and the term hamartoma of the spleen is considered acceptable. Both lymphoid and pulposal forms were represented in the group.

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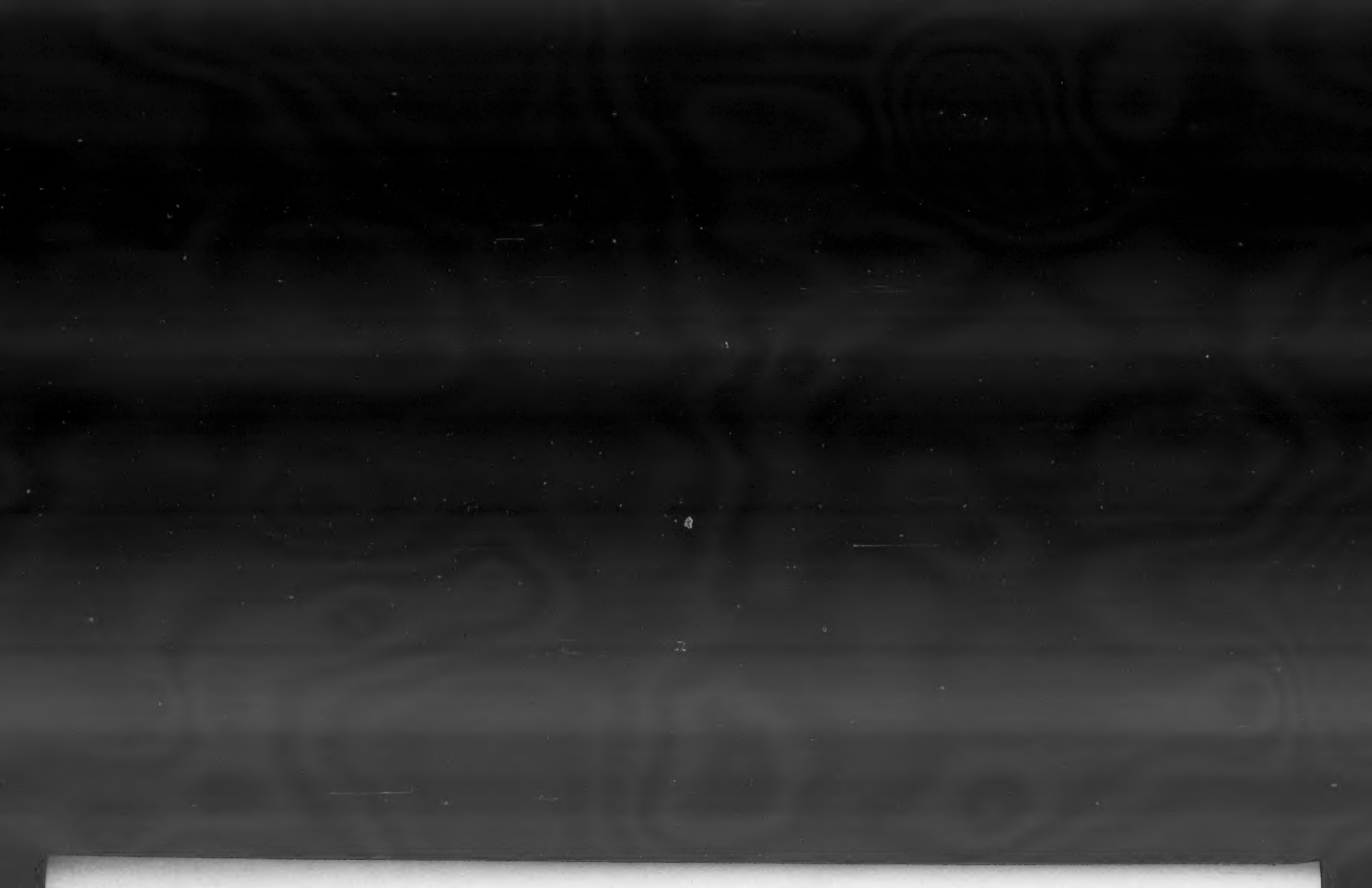
DESCRIPTION OF PLATES

PLATE 93

FIG. 1. Case 1. Gross specimen. The hamartoma is sharply demarcated from the surrounding spleen. This is accentuated by the hemorrhage which is dissecting the zone between the tumor and the normal parenchyma.

FIG. 2. Case 1. There is a marked compression effect adjacent to the tumor. Absence of capsule and presence of larger sinusoids within the tumor may be noted. × 20.

FIG. 3. Case 1. The cellular constituents of this tumor are almost completely lymphoid in character. × 250.



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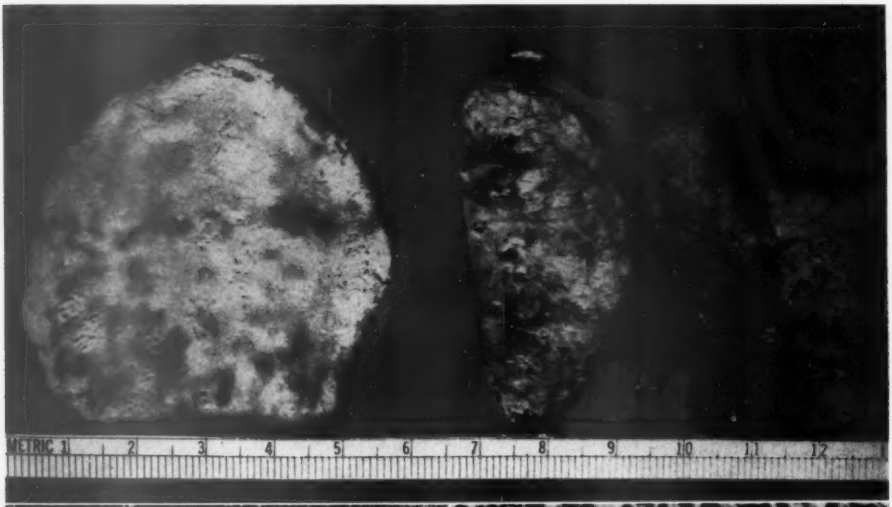

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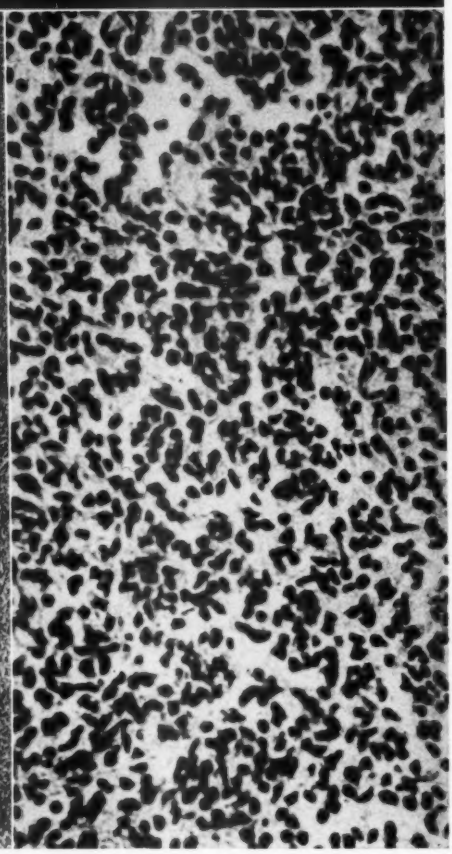


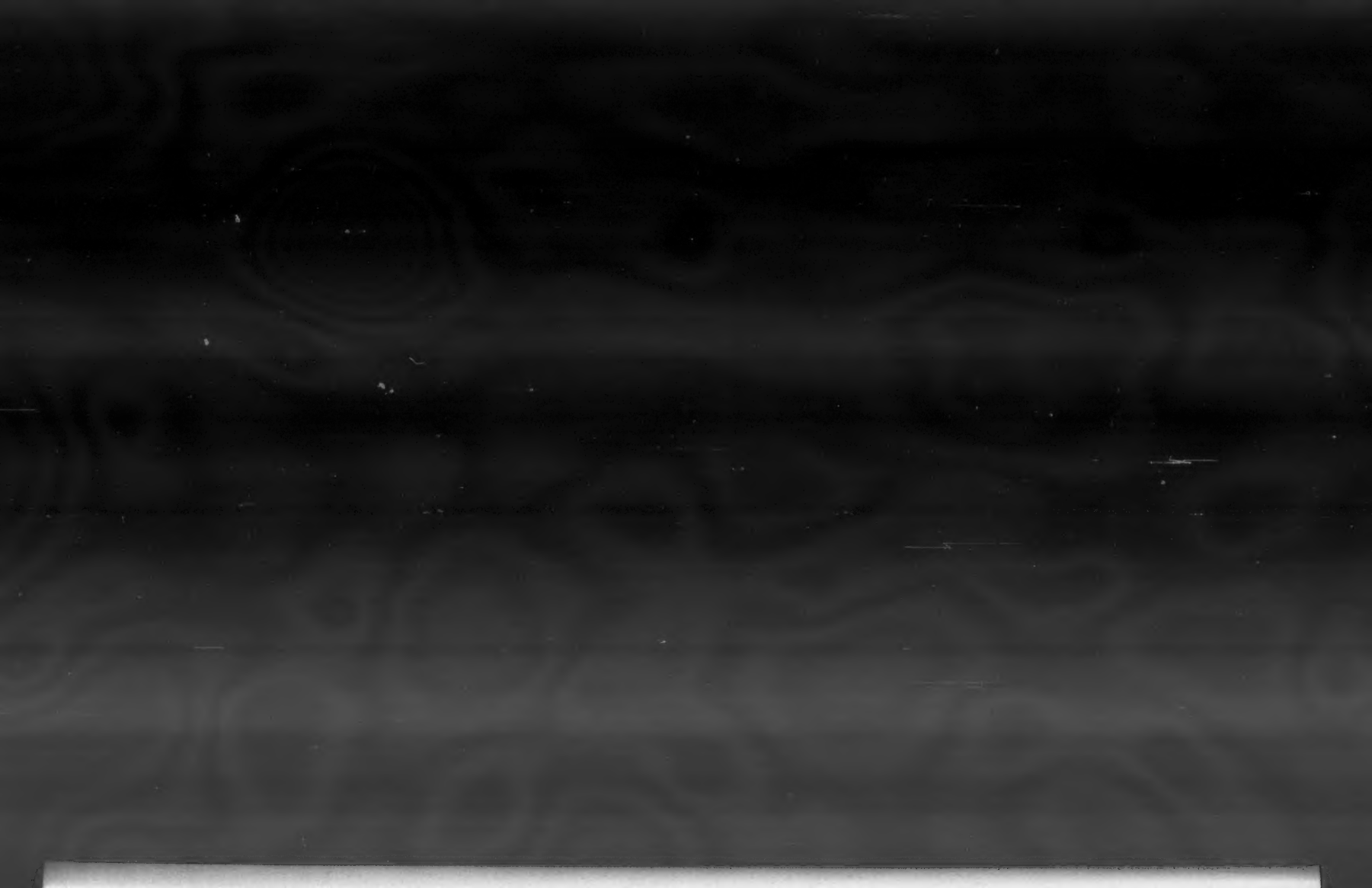
Coe and Von Drashek Hamartoma of the Spleen

PLATE 94

FIG. 4. Case 2. This field illustrates the similarity to normal red pulp. A focus of myeloid metaplasia is visible in the right half of the field. × 350.

FIG. 5. Case 3. Sinusoids are prominent. Pressure effect on normal spleen, in the lower half of the field, is less marked than in the preceding cases. × 20.

FIG. 6. Case 3. The lining cells of the sinusoids have the characteristics of littoral cells. The intersinusoidal tissue is more fibrotic and contains fewer free cells than the trabeculae in the surrounding splenic tissue. × 350.



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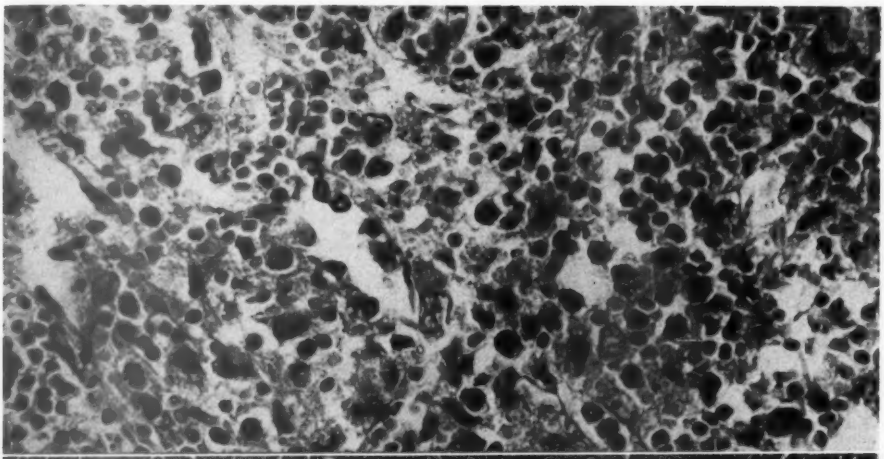

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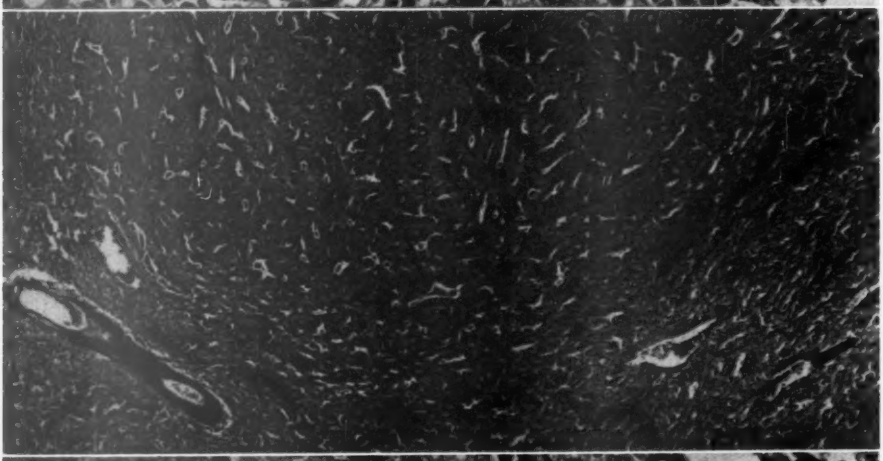

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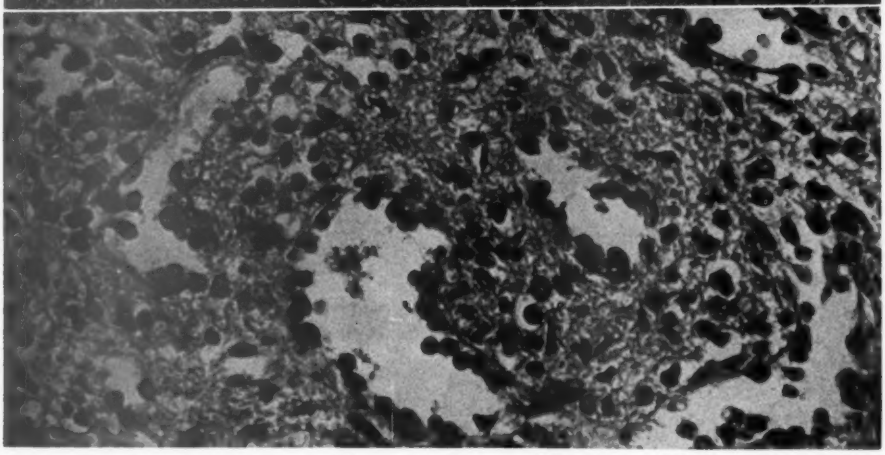

Coe and Von Drashek Hamartoma of the Spleen

OXYPHIL PARATHYROID ADENOMAS *

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Oxyphil parathyroid adenomas have generally been regarded as incidental pathologic curiosities encountered at necropsy and without practical clinical or endocrinologic importance. However, numerous scattered reports in the literature and our observations combined would indicate that this is frequently not the case.

Histologically, the parathyroid gland consists of pale and dark principal or chief cells, dark or transitional oxyphil cells, water-clear or wasserhelle cells, and pale or mature oxyphil (Welsh) cells, mingled with fat and blood vessels. It is the pale oxyphil cell adenoma with which this paper is mainly concerned. So-called transitional oxyphil cells are smaller, contain vacuoles, and resemble dark chief cells. They are frequently present in those parathyroid adenomas which usually produce hyperparathyroidism and which it is not the present purpose to discuss.

Grossly, the oxyphil adenoma is composed of soft, solid, gray-brown or yellow-brown tissue, not readily distinguishable from other parathyroid adenomas. Microscopically, the oxyphil cells are large, with abundant eosinophilic granular cytoplasm and small, regular nuclei. The cells have sharp borders and occur in sheets, cords, and occasionally form acini containing colloid. They contain little lipid, and the cytoplasmic granules take fuchsin stains. As in other parathyroid adenomas, mitotic figures are rare. To our knowledge, no acceptable oxyphil cell carcinoma of the parathyroid gland has been reported.

Including the first case as reported by Erdheim[1] (1903), a total of 25 cases of oxyphil adenoma have been recorded (Table I).[1-18] For 6 of the earlier cases the microscopic descriptions were incomplete and these are susceptible to other interpretations, but from the illustrations and text are considered acceptable oxyphil growths.

Six new cases of oxyphil adenoma have been added, including 3 observed at necropsy.

Case 1

F. F. (no. 132190) was a Jewish woman who had had 14 hospital admissions and had died at the age of 63 years. At 21 years of age, having borne 2 children, she had puerperal fever, and at 23 years, bilateral ectopic tubal pregnancies. Kidney gravel

* This investigation was supported by a research grant from the National Cancer Institute of the National Institutes of Health, Public Health Service.

Received for publication, February 29, 1952.

TABLE I
Reported Parathyroid Oxyphil Adenomas

Author	Sex	Age	Size	Site	Osteitis fibrosa cystica	Remarks
Erdheim[1] (1903)	M	18	2.5 cm.	RL		Healed rickets
Zehbe[2] (1909)						
Möller[3] (1911)	F	80	0.3 x 0.2 cm.			
Schmorl[4] (1912)						Osteitis deformans
Schmorl[4] (1912)	F	47			+	"Acromegaly," pituitary adenoma
Molineus[5] (1913)	F	59			+	
Molineus[5] (1913)	F	48	1.8 cm. 2.3 cm.	RL LL	+	Pituitary adenoma
Danisch[6] (1925)	M	72		LL		Diabetes mellitus, arteriosclerosis
Danisch[6] (1925)	M	71		LL		Carcinoma of stomach, obliterative pericarditis
Cushing and Davidoff[7] (1927)	M	35	0.9 cm. 0.4 cm.	RU LU		Acromegaly, cardiac failure
Hadfield and Rogers[8] (1932)	F	58	5 x 3.5 x 3 cm. (52 gm.)	L		Serum calcium, 11.8 mg.%
Gutman, Swenson, and Parsons[9] (1934)	F	60	2.0 cm. 1.5 x 0.7 x 0.4 cm.	RU RL	+	Polycystic kidneys, uremia; calcium, 10.2 mg.%
Lawrence and Zimmerman[10] (1935)	M	44	0.3 cm.			Cushing's syndrome; dissecting aneurysm
Warren and Morgan[11] (1935)	F	54	1.7 x 0.9 x 0.9 cm.	RU	+	Carcinoma of breast
Pappenheimer and Wilens[12] (1935)						4 cases, 2 of nephritis

Chown[15] (1937)	F	46	0.22 x 0.1 cm.			Ruptured cerebral aneurysm, hypertension, renal calculi
McQuillan[14] (1938)	F	46		R		Serum calcium, 10.2 mg.%; phosphorus, 4.2 mg.%
Cope[15] (1944)	F	70	6 x 3 x 3 cm. (23 gm.)			Died of cardiac failure; phosphorus, 3.8 mg.%
Norris[16] (1946)	F	23	3.5 cm.	L		Serum calcium, 12.3 mg.%
Willis[17] (1948)	F	55	2.5 x 1.5 cm.			Uterine fibroids, meningioma, adrenal cortical adenomas
Black and Ackerman[18] (1950)	F	59	3 x 3 x 1.3 cm.	LL	+	Hypertension, rheumatoid arthritis, nephritis; serum calcium, 10.6 to 13.8 mg.%; phosphorus, 3.9 to 6.3 mg.%
Black and Ackerman[18] (1950)	F	66	4.3 x 2.2 x 1.9 cm.	R		Died of renal insufficiency, renal calculi

R = right; RL = right lower; RU = right upper.
L = left; LL = left lower; LU = left upper.

was found when she was 30 years old. Eight years later a colloid goiter with multiple nodules and secondary hyperplasia was removed surgically. One year later hysterectomy and salpingo-oophorectomy were performed for adenomatous endometrial hyperplasia, adenomyosis, and leiomyoma. The ovarian cortical stroma showed marked hyperplasia and thecomatosis. There was postoperative hemorrhage. Pneumonia occurred at 47 years of age. The next year for the first time she developed hypertension of 170 to 190/88 mm. of Hg, and weight had increased from 145 to 165 lbs. Diabetes mellitus was diagnosed, requiring 18 units of insulin daily, but this the patient used erratically. At 57 years of age she survived perforating appendicitis with retrocecal abscess. Pyelitis was treated 3 years later. At age 62, a sigmoid cancer was resected with a low rectal anastomosis, because the patient refused abdominoperineal resection. Adenocarcinoma with blood vessel invasion and metastases to two of nine lymph nodes was diagnosed pathologically. The tumor recurred locally in 14 months and an abdominoperineal operation was performed, but the patient died the next day of atelectasis.

At necropsy, hypertensive heart disease, arteriolar nephrosclerosis, multiple mucosal polyps and submucous lipoma of the remaining colon, mesenteric lipoma, focal chronic pancreatitis, old cystic softening of the right basal ganglia, and cranial thickening consistent with old inactive Paget's disease were diagnosed.

Four parathyroid glands were isolated; they were not enlarged grossly, measuring from 0.2 to 0.5 cm. in greatest diameter.

One gland contained two sharply demarcated adenomatous nodules of oxyphil cells (Fig. 1), a cyst with colloid, and less well delimited areas of oxyphil cells. The other three parathyroid glands were within normal limits.

Microscopically, the pituitary body contained an eosinophilic adenoma measuring 0.7 by 0.5 cm.

Case 2

E. L. D. (no. 51A34) was 76 years old at death. A woman of Italian descent, she had had six successful pregnancies, and was first seen at age 70 because of 15 years of post-menopausal bleeding. She weighed 160 lbs., and had hypertension of 220 mm. of Hg systolic, with cardiac enlargement. On three occasions over the next 3 years specimens of endometrium taken for biopsy showed hyperplasia. There was estrogen effect apparent by vaginal smear and skin examination, but the source could not be found. The patient continued to have vaginal bleeding until her death from heart failure.

At necropsy, facial hirsutism was present. There were thrombi in the pulmonary arteries with pulmonary infarcts. A carcinoma simplex of the left adrenal cortex had metastasized to lymph nodes, right kidney, diaphragm, liver, and lungs. The endometrium showed adenomatous hyperplasia with foci of secretory activity, indicating endogenous production of both estrogen and progesterone, and there was cortical stromal hyperplasia of the ovaries. Cholelithiasis and arteriolar nephrosclerosis were found also. The head was not examined.

Three parathyroid glands were isolated. All were hyperplastic. In the largest gland there was a sharply encapsulated mass composed mainly of oxyphil cells, with a few included chief cells (Fig. 2). Foci of oxyphils were present in the other glands.

Case 3

E. V. H. (no. 36A12) was 68 years of age at death. A Canadian-born mother of 2 children, when 44 years old she had had a gastroenterostomy performed elsewhere for a large gastric ulcer eroding into the pancreas. At 57 years of age, appendectomy and removal of infarcted omentum were necessary. Two years before death a sore inside the left cheek was biopsied, with the diagnosis of epidermoid carcinoma grade II. The Hinton test was negative and blood pressure was 120/70 mm. of Hg. Many x-ray treatments were administered without arresting the extension of cancer to the maxilla and hard palate, with involvement of the tongue and death from terminal bronchopneumonia.

At necropsy, a mucinous adenocarcinoma was found in a 1.4 cm. gastric polyp, and there was also a 5 mm. pyloric peptic ulcer. Ovarian stroma showed cortical hyperplasia and granuloma formation. In the atrophic thyroid gland a 0.2 cm. oxyphil adenoma was present, compressing uninvolved parathyroid tissue composed of chief cells (Fig. 3).

Case 4

E. M. (no. 62214) was a woman of Irish extraction who had borne 3 children. At 33 years of age menopause was induced by radium in the treatment of an epidermoid carcinoma of the cervix. When she was 37 years old, removal of bladder stones was performed, and 10 years later an anomalous vessel to the right kidney producing hydronephrosis was cut. Because of a positive Hinton test, antiluetic therapy was administered for 2 years at 38 years of age. When she was 49 years old abdominal pain and gastro-intestinal roentgenograms led to a diagnosis of duodenal ulcer, which was treated by milk, cream, and amphojel for 2 years. At 51 years of age, roentgenograms showed increased density of the skull with mottled and punched out areas, fuzziness of the vertebrae, and coarse trabeculation of the innominate bones. Diagnosis was difficult, but repeated chemical examinations of the blood revealed serum calcium, 12.5 to 13.0 mg. per cent; phosphate, 2.0 to 5.0 mg. per cent; alkaline phosphatase, 15.8 to 16.9 Bodansky units; and Sulkowitch test, 3 plus. After 6 months, further roentgenograms showed cystic changes in the long bones, considered indicative of hyperparathyroidism and possibly also of Paget's disease. The urine contained Bence Jones protein, but marrow puncture showed only hyperplasia.

Operation at 52 years of age resulted in the removal of a right lower parathyroid adenoma, 3.5 by 2 by 1.5 cm., and 25 gm. of colloid goiter with multiple nodules. Postoperative roentgenograms showed increased bone density, confirming pre-existing hyperparathyroidism. After operation the patient suffered from hypertension of 180/110 mm. of Hg and synovitis. Six years postoperatively, at 58 years of age, she died of progressive bulbar muscular atrophy.

Microscopically, the parathyroid tumor was composed chiefly of interdigitating cords of large oxyphil cells, which made up 80 per cent of the gland (Fig. 4). Necropsy elsewhere showed in addition to muscular atrophy, cardiac hypertrophy and dilatation of hypertensive type, pulmonary congestion and edema, fibrous pleuritis, moderate hydronephrosis, a 1.5 cm. cortical adenoma of the right adrenal gland, and radiation damage to the cervix and bladder with ulcerative cystitis.

Case 5

M. A. (no. 104582) was 31 years old and mother of one child. She had had an x-ray diagnosis of a cyst of the mandible 1 year previously, and 5 months previously three more cysts had developed in the jaw and one in the pubic bone. Calcium stones were found in the kidneys. An operation was performed at this time in another hospital without discovery of a parathyroid tumor. Chemical findings later included serum calcium, 14.5 per cent; phosphorus, 3.9 mg. per cent; alkaline phosphatase, 4.8 Bodansky units; Sulkowitch test, 2 plus. Roentgenograms showed bone changes typical of hyperparathyroidism and tiny renal calculi. At the second operation a tumor 2 by 2 by 0.9 cm. was removed from the lower right area of the neck.

Microscopically, the tumor had a thick fibrous capsule with delicate trabeculae and was composed in part of solid areas of oxyphil cells and elsewhere of mixed oxyphils and dark chief cells (Fig. 5).

Case 6

L. B. O. (no. 128085) was a woman who had had three pregnancies and two successful deliveries. When she was 59 years old she had been seen because of excessive vaginal bleeding for 2 years, previous to which she claimed normal menses. She weighed 202 lbs. and had hypertension of 210/100 mm. of Hg. By biopsy, a patho-

logic diagnosis of epidermoid carcinoma of the cervix was made. Successful radiation therapy was given. At 67 years of age a cyst of the right kidney was removed. At 69 years she developed diabetes mellitus. One year later she was seen with a basal metabolic rate of plus 24, a nodular thyroid gland, auricular fibrillation, and loss of weight to 149 lbs. At operation a colloid goiter with multiple nodules was removed. Posterior to the right thyroid lobe was a soft, yellow-brown, 0.9 cm. nodule; coincidentally with its manipulation the systolic blood pressure dropped from 170 to 70. The nodule was removed, and proved microscopically to be an oxyphil parathyroid adenoma with admixture of dark chief cells (Fig. 6). A normal parathyroid gland also was identified.

Of the 6 cases reported, all were in married women with children. Five were over 50 years of age. Two had evident hyperparathyroidism, 2 possibly Paget's osteitis deformans, 2 diabetes mellitus, and 5 had malignant neoplasms—carcinoma of cervix (2), sigmoid colon, adrenal cortex, mouth, and stomach—one with multiple primary cancers. Of the 4 cases without manifest parathyroid hyperfunction, one had kidney stones. Nodular goiters were present in 3, with secondary hyperplasia in 2. Endometrial hyperplasia was present in 2 patients. Hypertension was found in 4.

The frequent combinations of physiopathologic changes of a parathyroid gland, thyroid gland, pancreas, and uterus suggest a polyglandular syndrome and make one suspect pituitary dysfunction. Studies by others of acromegaly, Cushing's syndrome, and other hyperpituitary states already have shown a surprising incidence of parathyroid hyperplasia and adenoma formation (Table II). Additional cases[35-39] have been collected which, on anamnestic and morphologic grounds, have been thought to indicate that parathyroid hyperplasia or adenomas may form part of a polyhormonal pituitary disorder, particularly in women. Our material would support this thesis.

It has been asserted on good authority that oxyphil adenomas are non-functioning and do not produce hyperparathyroidism.[18,40] Yet 6 cases reported previously and 2 presented here have had osteitis fibrosa cystica, kidney stones, elevated serum calcium, or a combination of these abnormalities usual in parathyroid hyperfunction. It would appear sound to conclude that while not the most common source of hyperparathyroidism, oxyphil adenomas can exhibit hyperfunction. Assumption of the existence of a small, hidden, active chief cell or water-clear cell adenoma seems unjustified. However, in agreement with the belief that the oxyphil is an involuted cell form,[40] it may be that its adenomas are relatively quiescent or "burned out" when removed and examined.

For comparison, 28 adenomas of more common parathyroid types were re-examined microscopically. Of these, 25 were mainly of chief

cell type, and 3 of water-clear cells. No oxyphils were identified in 13, rare scattered oxyphils in 11, and from a few to moderate numbers in 4 adenomas.

One unusual tumor of the parathyroid region was found in a man, 57 years old, suffering from slight difficulty in swallowing and desire to clear his throat. At operation 110 gm. of colloid goiter with multiple nodules was removed. A mass 1.5 cm. in diameter was resected from the right lower thyroid pole, and considered to be a parathyroid adenoma. A normal parathyroid gland was removed also. On the day of operation serum calcium was 9.2 mg. per cent; phosphorus, 4.1 mg. per cent; and phosphatase, 3.8 Bodansky units. There was slight postoperative tetany, with calcium falling to 5.6 mg. per cent. Microscopically, the 1.5 cm. nodule proved to be a granular cell myoblastoma (Fig. 7).

Myoblastoma cells differ from parathyroid oxyphil cells in their irregular shapes and larger size. Prominent eosinophilic cytoplasmic granules of the myoblast cells are characteristic. Adjoining cells have the cross striations characteristic of voluntary muscle. Eosinophilic cells occur also in other cervical neoplasms, particularly in carotid body tumors and Hürthle cell adenomas of the thyroid gland. Microscopically the pattern of the carotid body tumor is vascular and glomangioid, accentuated by silver stains. The perivascular pink cells are quite irregular in size and shape, with clear nuclei, prominent nucleoli, and little resemblance to epithelium. Hürthle cell adenomas are mostly sharply encapsulated, relatively large intrathyroid tumors. Histologically the Hürthle adenoma pattern is relatively organoid or acinar, contrasting with the pavement-like polygonal parathyroid oxyphil cells. Hürthle cells are larger with more prominent vesicular nuclei. Colloid is more easily found in Hürthle cell tumors, and scanty in parathyroid adenomas.

Discussion

One objective of this presentation has been to indicate that oxyphil parathyroid adenomas may occur with hyperparathyroidism. Other such tumors had no evident function, although some gave hints of antecedent activity by slight hypercalcemia or kidney stones. They occurred predominantly in women (20 of the 25 cases in which the sex was stated). All but 5 patients were over 45 years old. Because of their relatively small size, oxyphil adenomas are not often discovered except by careful surgical or post-mortem exploration, but are probably not rare.

Based upon our experience, we believe that persons with oxyphil parathyroid adenomas frequently show indications of more generalized

TABLE II
Abnormal Parathyroid Glands in Hyperpituitarism

Author	Sex	Age	Size of parathyroid glands	Parathyroid glands, microscopic findings	Pituitary body	Other findings
Erdheim[1] (1903)	M	42	RU 1.2 x 0.5 x 0.3 cm. LU 0.8 x 0.8 x 0.3 cm. RL 1.1 x 0.6 x 0.4 cm. LL 1.7 x 0.6 x 0.3 cm.	3 hyperplastic	Eosinophil adenoma (acromegaly)	Aortic insufficiency
Claude and Baudouin[19] (1911)	F	51	(5 or 6 times normal)	Hyperplasia, oxyphil cells and colloid	Eosinophil adenoma (acromegaly)	Thyroid and adrenal hyperplasia
Schmorl[4] (1912)	F	47	(Enlarged)	Oxyphil and chief cells	Basophil adenoma (Cushing)	Osteitis fibrosa cystica, hirsutism, obesity
Carnot, Rathery, and Dumont[20] (1913)	F	58	0.8 x 0.3 cm. 0.8 x 0.3 cm. (3 times normal)	Hyperplasia, chief and oxyphil cells	Acidophil adenoma (acromegaly)	Splanchnomegaly
Molineus[5] (1913)	F	48	1.8 cm. 2.3 cm.	Hyperplasia and oxyphil adenomas	Basophil adenoma (Cushing)	Scar of peptic ulcer, thyroid gland enlarged, obese
Harbitz[21] (1915)	M	75	1 x 1.2 cm. 2 x 2.5 cm.	Multiple adenomas	Eosinophil hyperplasia	Splanchnomegaly
Josefson[22] (1915)*	M	26	6 x 4 x 2.5 cm.	Adenoma, oxyphil(?)	Chromophil adenoma (acromegaly)	Splanchnomegaly
Raab[23] and Kraus[24] (1924)†	M	31	Together, 9.16 gm.	Large nests of oxyphil cells	Basophil adenoma (Cushing)	Obese
Cushing and Davidoff[7] (1927)	M	52	1 x 0.8 x 0.5 cm.	Hyperplasia	Eosinophil adenoma (acromegaly)	Adenomas of thyroid gland and adrenal cortex
	M	35	0.9 cm. 0.4 cm.	Chief cell and oxyphil adenoma; oxyphil adenoma	Eosinophil hyperplasia (acromegaly)	Adenomas of thyroid and adrenal glands, and pancreas
Lloyd[25] (1929)	F	22	LU 0.3 cm. RL 1.5 x 0.4 x 0.3 cm. LL 1.7 x 1.0 x 0.8 cm.	Negative Hyperplasia of oxyphil and clear cells	Eosinophil adenoma (acromegaly)	Pancreatic islet adenomas

Hadfield and Rogers[8] (1932)	M	51	8 x 4 x 2.5 cm.	Chief cell adenoma	Enlarged (acromegaly)	Kidney gravel, thyroid adenomas
Hoff[26] (1934)	M	16	Generalized enlargement	Not given	Basophil adenoma	Osteitis fibrosa cystica, calcinosis
Hora[27] (1935)‡	F	38	Enlarged, RL 5 times normal	Not given	Basophil adenoma (Cushing)	Adrenal cortical hyperplasia and adenoma
Minciotti[28] (1935)‡	F	38	Not given	Hyperplasia	Non-granular adenoma (Cushing)	Osteoporosis; adrenal, thyroid hyperplasia
Lawrence and Zimmerman[10] (1935)	M	44	0.3 cm. 0.3 cm.	Adenoma, atrophic (type?)	Basophil adenoma (Cushing)	Adrenal adenomas, dissecting aneurysm
Kalbfleisch[29] (1937)	M	23	RU, LL, RL: "pea sized" LU larger	Hyperplasia, chief cell and oxyphil (?) adenomas	Invasive chromophobe adenoma	Obesity, 5 pancreatic islet adenomas
Franck and Hjerrild[30] (1937)	F	63	RL 4.5 x 2.5 x 1.5 cm. 3 x 1.5 x 1 cm. LL 2.0 x 0.8 x 0.6 cm.	Bilobed chief and clear cell adenoma Clear, chief, and oxyphil cell adenoma	Basophil hyperplasia	Osteitis fibrosa cystica, thyroid adenoma, fibrosed ovaries
Gerstel[31] (1938)	M	36	RL "egg size"	Chief cell adenoma	Eosinophil adenoma (acromegaly)	Pancreatic tumor, nodular adrenal and thyroid glands
Black and Ackerman[18] (1950)	M	60	Sup. mediastinum, 4 x 3 x 3 cm.	Chief cell adenoma	Eosinophil adenoma	Pancreatic islet adenomas; nodular adrenal and thyroid glands
Sprague *et al.*[32] (1950)	M	15	RL	Chief and transitional cell adenoma	Not examined (clinically, Cushing)	Adrenal cortical and thymic hyperplasias, thymic adenoma

* Cited by Cushing and Davidoff.[7]
† Cited by Cushing.[33]
‡ Cited by Kessel.[34]

metabolic disturbances. Pregnancy has been claimed as a condition in which pituitary activity favors initiation of parathyroid hyperfunction.[38] Peptic ulcers, now regarded by some as by-products of pituitary adrenocorticotropin overproduction following stress, also were seen in our cases and are occasional precursors of other types of parathyroid disease. In so-called primary parathyroid hyperplasia, clinical and metabolic studies have indicated possible participation of pituitary hormones.[41] Parathyroid hyperplasia and adenoma formation secondary to anterior pituitary stimulation have been tabulated in the attested cases of acromegaly and Cushing's disease. Other less clear-cut hyperpituitary states, including some cases of Morgagni-Stewart-Morel syndrome,[35] also have shown evidence of hyperparathyroid function.

Since acromegaly, Cushing's pituitary basophilism, and less specific anterior lobe hyperplasias are all implicated, the cellular source of the stimulus cannot be identified accurately.[39] However, the cases collected indicate human pituitary-parathyroid gland relationships more intimate and important than those suggested by animal experiments.[42]

The 6 new cases reported had no characteristic clinical endocrine stigmata, but rather a variety of diseases and hyperplastic states such as diabetes mellitus, endometrial hyperplasia, ovarian cortical stromal hyperplasia, adrenal cortical adenoma or carcinoma, hypertension, and nodular goiters with hyperthyroidism. Various workers have considered anterior pituitary stimuli important in the development of each of these conditions.

As has been repeatedly pointed out, no satisfactory criteria are available to distinguish with certainty between adenoma and localized hyperplasia of the parathyroid gland. Suggestive indications of a benign neoplasm include the localized homogeneous character of growth and giant cells of tumor type. Nodular hyperplasia has qualities merging with adenoma in the parathyroid gland as in adrenal and pituitary glands. This may indicate that in these tissues such morphologic distinctions are of only secondary importance.

If one accepts the tenet that all parathyroid cells are of a single type, with different appearances in varying functional states, oxyphil cells seem most comparable to involuted forms,[40] such as Hürthle cells of the thyroid gland and onkocytes of salivary glands. Oxyphil adenomas in general are removed in a condition of waning or spent function. They are considered to connote likelihood of past or present hyperparathyroidism, and to be morphologic evidence of anterior pituitary hyperactivity of varying type, degree, and duration. Besides the production of parathyroid adenomas, pituitary stimuli appear to

have initiated concurrent pathologic changes in various other susceptible tissues. Other etiologic factors of major importance doubtless exist in some parathyroid adenomas, and in the absence of a better understanding of the hormones involved, the suspected causes and effects remain unproved.

SUMMARY

Six cases of parathyroid oxyphil adenoma are reported, in addition to 25 collected from the literature. Eight patients had osteitis fibrosa cystica and evident hyperparathyroidism. Usually oxyphil adenomas appear to be a less active, involuted type of parathyroid growth. Evidence collected from the cases presented and the literature shows that anterior pituitary hyperfunction may contribute to parathyroid hyperplasia or adenoma formation. Some parathyroid adenomas occur as part of a polyglandular endocrine syndrome. Human pituitary-parathyroid gland relationships appear to warrant an increased emphasis.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

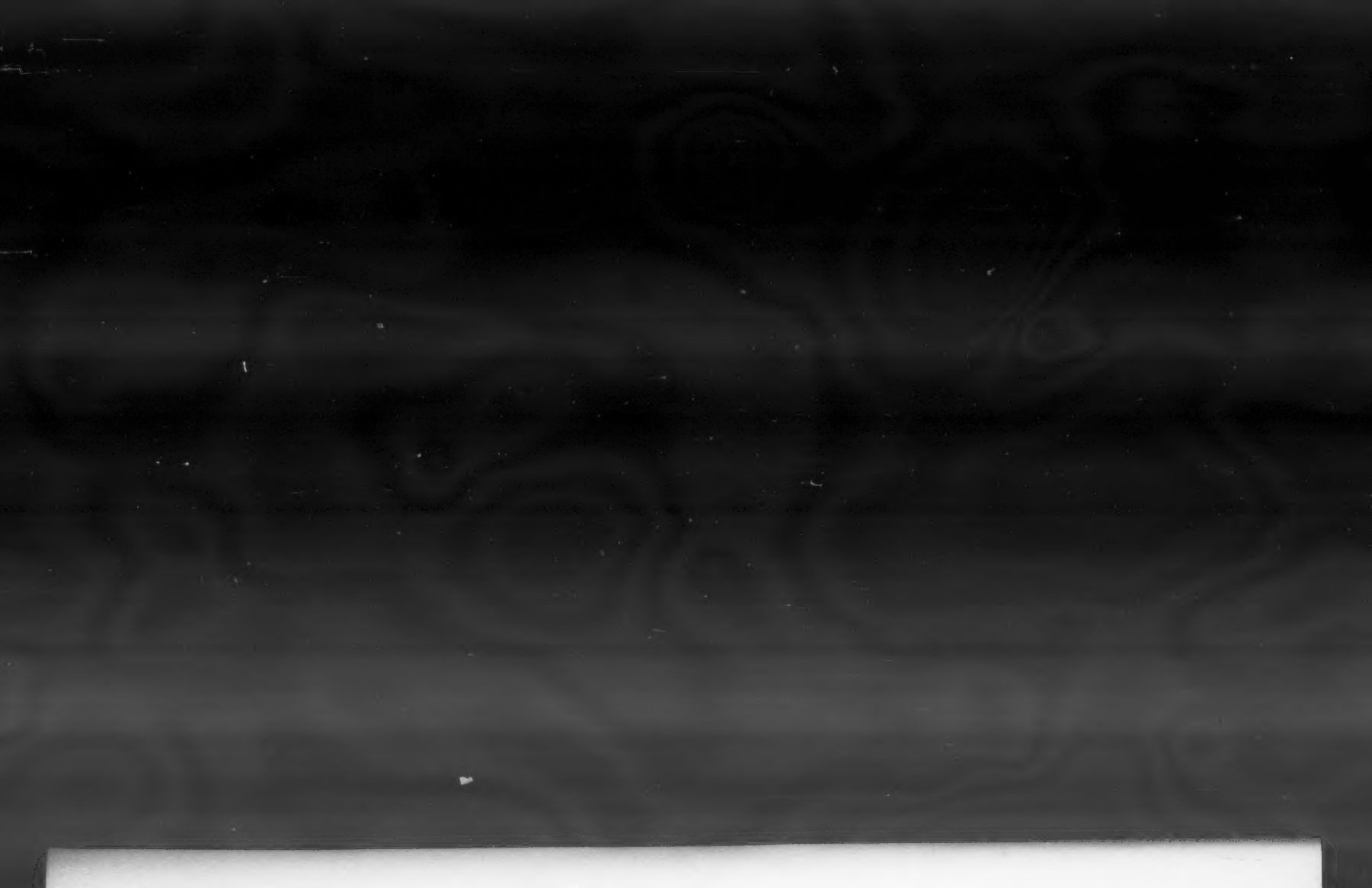
PLATE 95

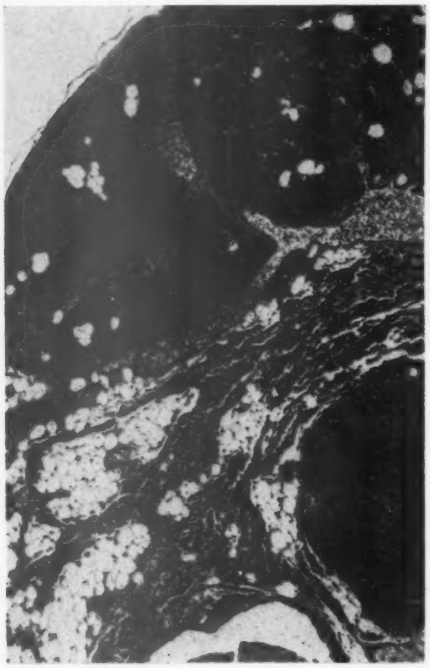
FIG. 1. Case 1. Two adenomatous oxyphil parathyroid nodules. Aside from one small cyst, the parathyroid glands were otherwise normal. Hematoxylin and eosin stain. × 50.

FIG. 2. Case 2. Portion of a solid encapsulated oxyphil adenoma of the parathyroid gland. Hematoxylin and eosin stain. × 150.

FIG. 3. Case 3. Intrathyroid parathyroid oxyphil adenoma. Hematoxylin and eosin stain. × 50.

FIG. 4. Case 4. Characteristic interwoven cords of large cells with granular eosinophilic cytoplasm, from an oxyphil parathyroid adenoma. Eosin and methylene blue stain. × 300.





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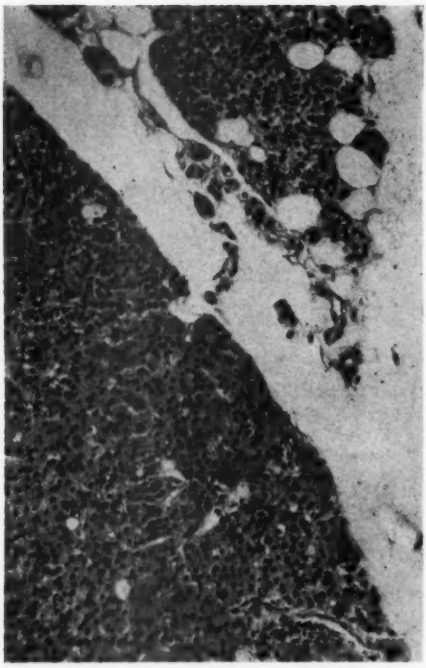

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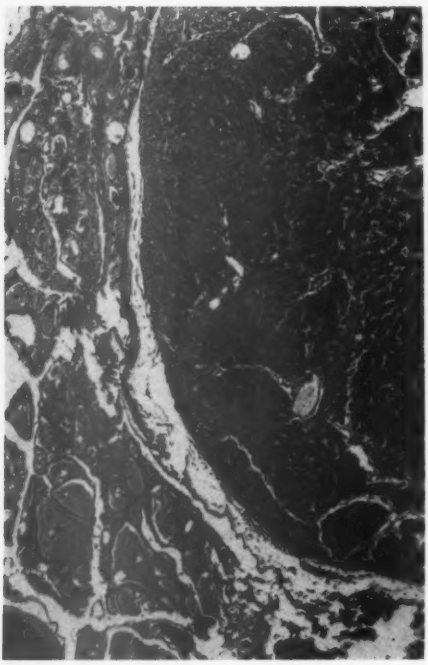

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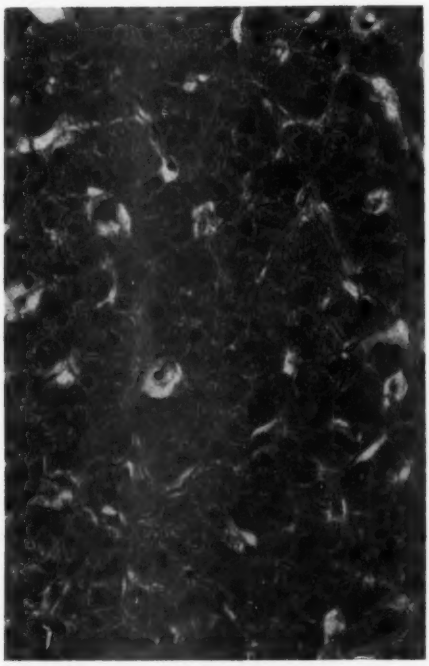


4

Sommers and Young

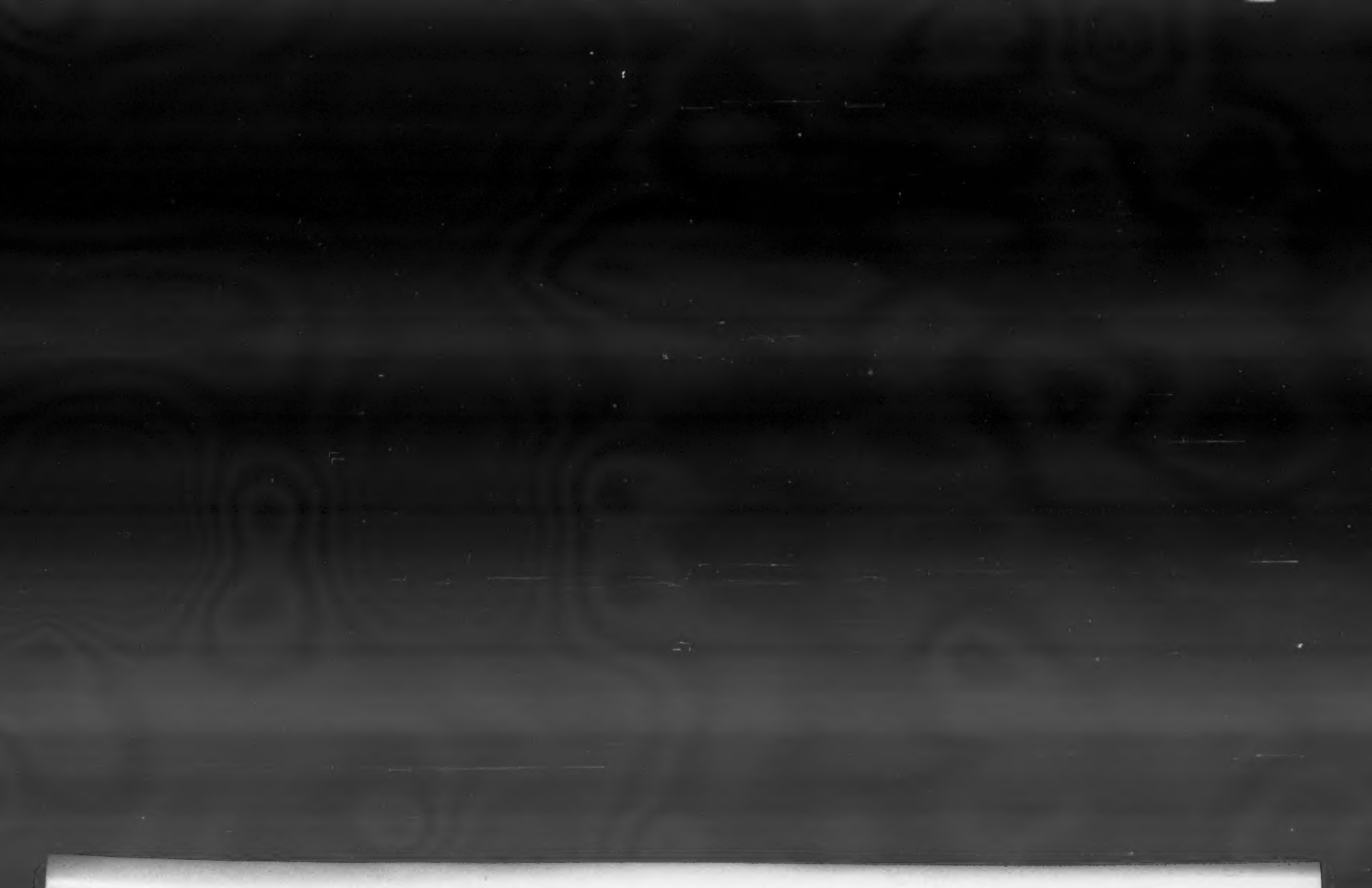
Oxyphil Parathyroid Adenomas

PLATE 96

FIG. 5. Case 5. Solid oxyphil adenoma of the parathyroid gland. Eosin and methylene blue stain. × 300.

FIG. 6. Case 6. A part of the parathyroid adenoma demonstrating intermingled oxyphil and chief cells. No clinical hyperparathyroidism was observed. Hematoxylin and eosin stain. × 300.

FIG. 7. Part of the granular cell myoblastoma removed from the parathyroid region. Cytoplasmic granularity and suggestive striations are present. Cells are much larger than parathyroid oxyphil cells. Hematoxylin and eosin stain. × 300.



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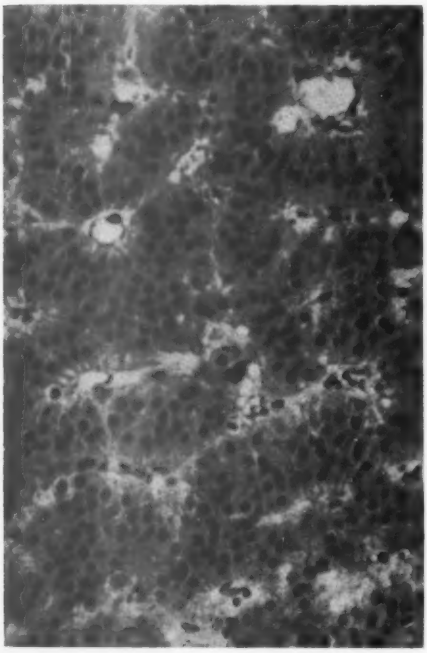

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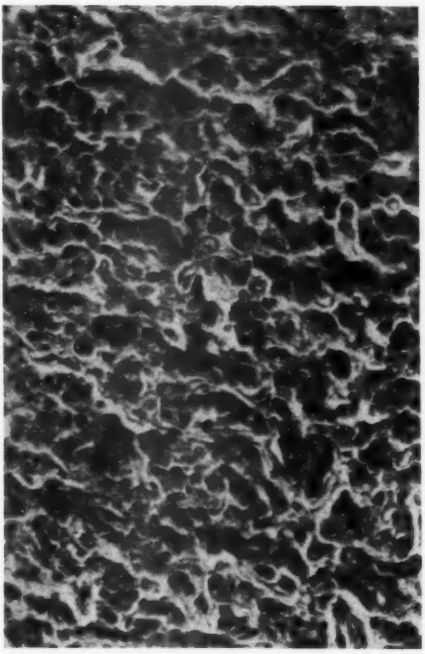

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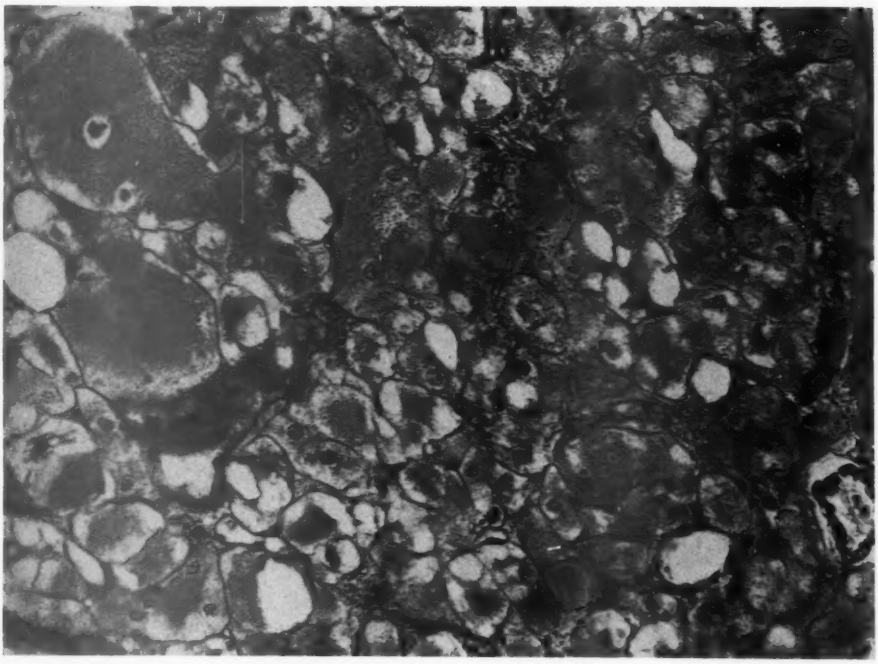

Sommers and Young Oxyphil Parathyroid Adenomas

CLEAR CELL MYO-EPITHELIOMA OF THE SKIN

Report of Ten Cases *

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This communication describes the histologic features of a benign cutaneous tumor of which 10 specimens have been observed at the Massachusetts General Hospital in the past 20 years. In spite of its distinct histologic appearance, characterized by the presence of numerous clear epithelial cells, reports about this tumor are rare.

Liu[1] reported 4 tumors which he called clear cell papillary carcinoma, although no metastases were observed. They were composed of clear cuboidal-shaped epithelial cells arranged in a papillary manner. Abundant amounts of glycogen were demonstrated with Best's carmine stain in both the clear and non-clear cells. Liu thought that the tumor was derived from hair follicles and represented a variety of basal cell carcinoma. Stout and Cooley[2] described a tumor characterized by "the formation of squamous epithelial cells of a specialized type with clear swollen cytoplasm" and interpreted it as a papillary cystadenoma of sweat glands. They believed that it was not a true carcinoma and called it sweat gland epithelioma with clear cells.

Our studies have not led to a definite conclusion as to the histogenesis of this tumor, but the evidence points to a myo-epithelial sweat gland tumor.

Clinical Data

The 10 tumors were all solitary, had been present from 1 to 5 years, and were located on the scalp, neck, arms, legs, pubic region, and vulva. The patients varied in age from 14 to 65 years; 2 were male; 8, female.

The clinical appearance of the lesion was that of a solid nodule, although in one patient the tumor felt cystic. Except for 3 cases in which the tumor discharged serous material, the epidermis overlying the tumor was intact. In 2 patients the lesion had a bluish color so that a clinical diagnosis of pigmented nevus had been made in one.

None of the tumors had given rise to metastases and no instance of recurrence after excision was recorded.

Histologic Description

In all 10 patients the tumor was located in the corium fairly close to the epidermis and was composed of irregularly shaped and variously

* Received for publication, February 29, 1952.

sized lobular masses of epithelial cells (Fig. 1). Some of the lobules lay closely packed, while others were separated by strands of fibrous tissue. Although in general well demarcated, none of the tumors was encapsulated and most of them showed small outlying lobules of neoplastic tissue. In 7 tumors no connections of the epithelial masses with the surface epidermis were noted; in 2 the tumor masses had reached the epidermis which, in turn, showed irregular hyperplasia and downward growth; and in one the tumor masses had largely replaced the epidermis overlying the tumor.

All tumors showed epithelial cells of at least two types (Figs. 2 and 5): an elongated, often fusiform cell with basophilic cytoplasm and a large, deeply basophilic, elongated nucleus similar to the myo-epithelial cells seen in myo-epithelioma; and a round or cuboidal cell with a distinct cellular membrane, very clear cytoplasm, and a round nucleus. The latter cells, the presence of which gives the tumor its characteristic appearance, seemed to develop from the myo-epithelial cells. Due to the fact that the clear cells had a greater amount of cytoplasm than the basophilic cells, the nuclei of the clear cells lay farther apart and in thin sections some of the clear cells showed no nucleus at all. Of the 3 cases that were examined for glycogen (Hotchkiss reaction[3]), 2 showed large amounts and one a small amount of glycogen in the clear cells (Figs. 6 and 7).

Additional findings in some of the tumors were the presence of horny cells, glandular structures, and cysts. In 5 tumors typical squamous cells, with distinct intercellular bridges and areas of cornification, were seen (Figs. 3 and 4). Cornification manifested itself either as true horny pearls or as sheets of cornified material.

In 6 tumors distinct tubular lumina lined by glandular epithelium were present. The cells of the glandular epithelium showed in several instances active secretion of the type referred to as decapitation secretion by Schiefferdecker[4] and regarded as typical of apocrine sweat glands. The glandular lumina were generally small (Fig. 8), but in 2 tumors fairly large, branching lumina were observed (Fig. 9).

Whereas clinically only one tumor gave the impression of being cystic, gross examination of the specimen revealed cysts large enough to be visible with the naked eye in all but one case. These cysts were the result of disintegration of tumor cells. In several cases large lobules had undergone almost complete cystic degeneration leaving only a thin wall of degenerating epithelial cells. In 2 cases some of these cells showed mucoid degeneration (Fig. 10). Smaller cysts were, in some instances, densely filled with degenerated clear cells which had lost their nuclei (Fig. 11).

Discussion

The histogenesis of these tumors poses a difficult question. The presence of tubular gland lumina in some of these tumors suggests an origin from sweat glands, probably apocrine sweat glands because of the presence of active secretion. The elongated basophilic cells certainly resemble myo-epithelial cells and, since myo-epitheliomas are characterized by the presence of tubular lumina embedded in masses of irregularly proliferating myo-epithelial cells,[5,6] it seems likely that the tumor is a myo-epithelioma. The absence of tubular lumina in some of the tumors would not militate against this concept of origin since the important cell is the myo-epithelial cell.

Although none of the cases of myo-epithelioma described in the literature have shown clear cells, cyst formation, or cornification, it is believed that these merely represent minor, though conspicuous, variations. As to the presence of clear cells, cells in other tumors and organs may also show extreme vacuolization of their cytoplasm. For example, squamous cell carcinoma of the cervix may become so vacuolated that it simulates a hypernephroma. The parathyroid chief cell, especially when filled with glycogen, becomes very clear and is then referred to as a water-clear cell. The formation of cystic cavities and the mucoid changes in some of the epithelial cells represent only a degeneration such as occurs in many types of tumors. The presence of squamous and horny cells is admittedly unusual in tumors of glandular origin. Stout and Cooley,[2] considering the tumor a sweat gland epithelioma, explained the presence of keratinization in these tumors as "epidermoid metaplasia." On the other hand, Liu[1] regarded the presence of cornification as an attempt at hair shaft formation in conformity with his idea that the tumors are derived from hair follicles. Perhaps this decision was facilitated for him by the fact that none of his 4 tumors contained tubular lumina.

An interesting aspect is the presence of glycogen in some of the clear cells of the tumor. Since in the normal skin the outer hair sheath is the only structure which contains a significant amount of glycogen,[7,8] the presence of glycogen in the clear cells of the tumor was the main reason why Liu[1] believed these tumors were derived from the outer hair sheath cells. On the other hand, glycogen is commonly found in young cells, and therefore it would be hazardous to attribute too much significance to its presence in the tumor cells.

Summary

Ten examples of a benign cutaneous tumor presented a distinct histologic appearance characterized by lobules composed of epithelial

cells of at least two types: myo-epithelial cells and glycogen-containing clear cells. In addition, some tumors contained squamous and horny cells, tubular lumina lined by glandular cells, and cysts. The histogenesis is still obscure. It is probable that this tumor represents a variation of myo-epithelioma.

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DESCRIPTION OF PLATES

PLATE 97

FIG. 1. Low magnification reveals the lobular architecture of the tumor. On the left is part of a large cyst. × 90.

FIG. 2. Higher magnification shows the two types of cells: (1) elongated basophilic myo-epithelial cells and (2) clear cells. × 180.

FIG. 3. Three horny pearls are present in the area illustrated. × 200.

FIG. 4. An area of cornification is surrounded by squamous cells. × 400.

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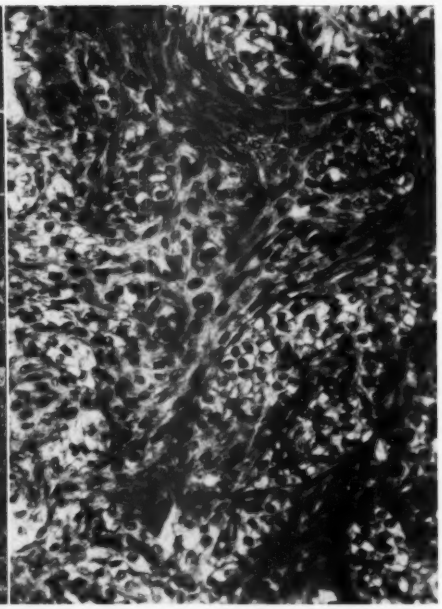

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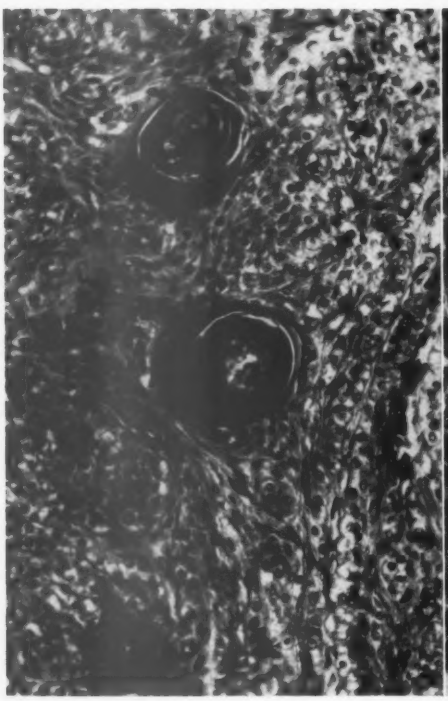

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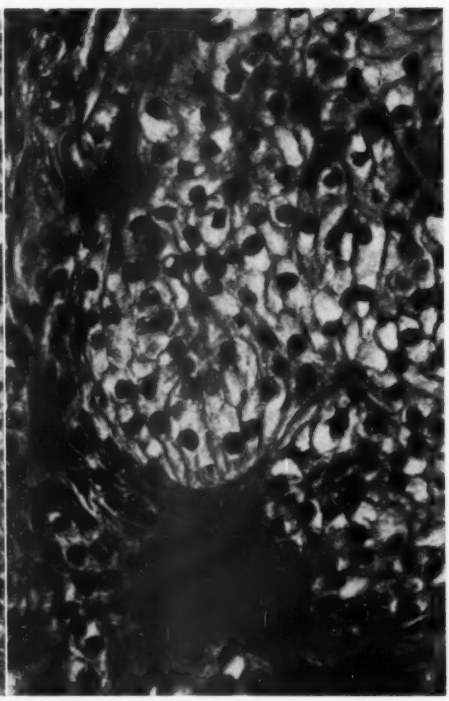

4

Lever and Castleman

Clear Cell Myo-epithelioma

PLATE 98

FIG. 5. A still higher magnification than in Figure 2 shows the vacuolar character of the clear cells. × 400.

FIG. 6. The clear cells of the tumor contain considerable amounts of glycogen as shown by a strongly positive Hotchkiss[3] reaction before exposure of the section to saliva. × 400.

FIG. 7. After exposure of the section to saliva the reaction is negative. × 400.

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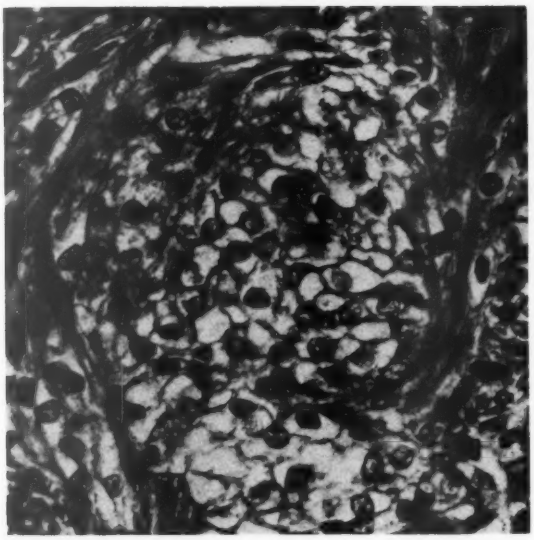

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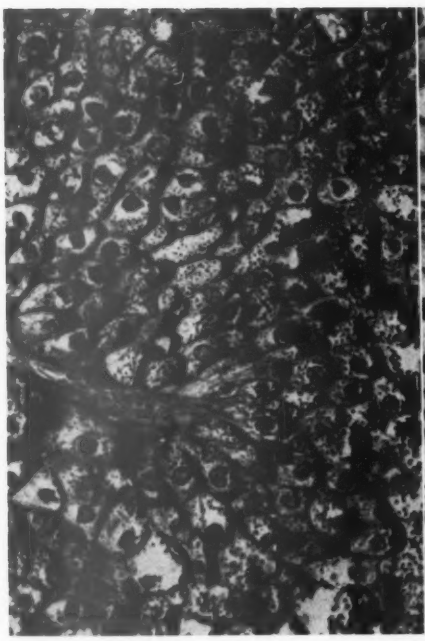

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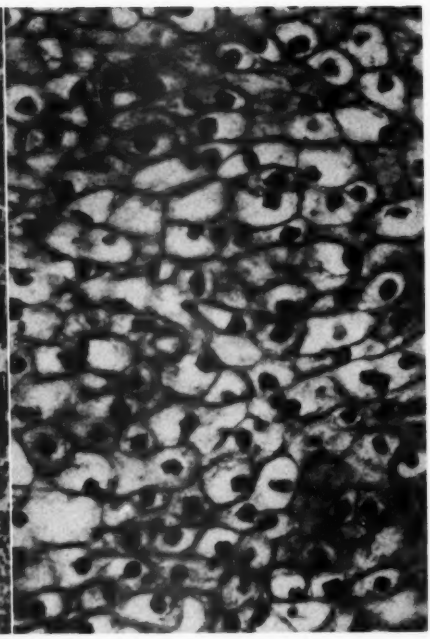


Lever and Castleman

Clear Cell Myo-epithelioma

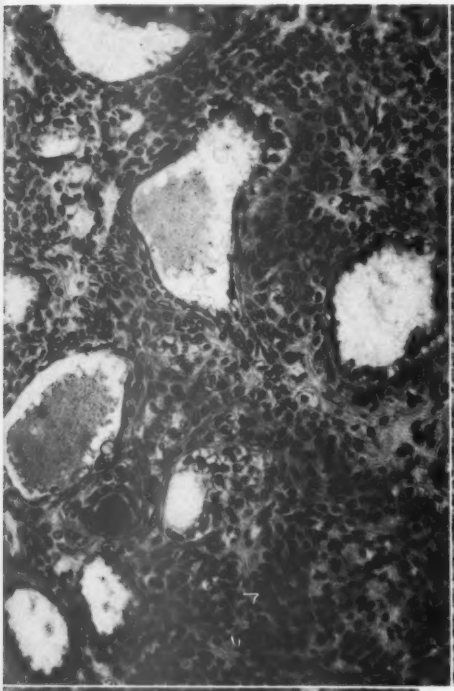
PLATE 99

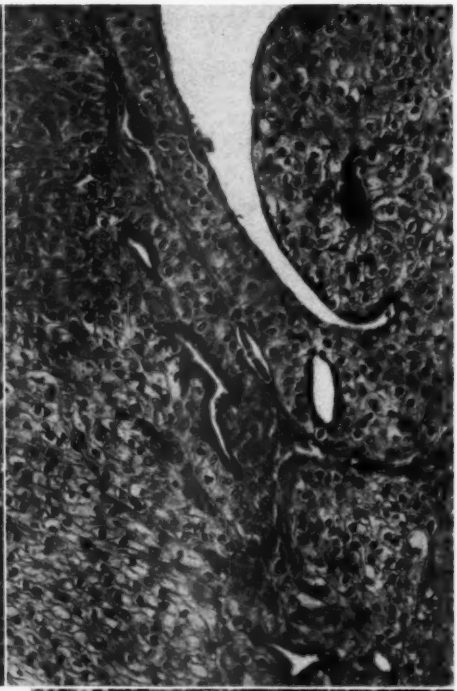
FIG. 8. There are several small tubular lumina lined by actively secreting cells. The secretory activity suggests that they are apocrine gland lumina. × 200.

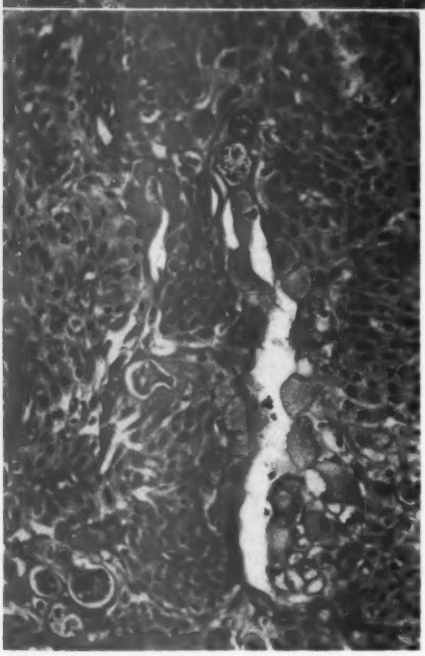
FIG. 9. Several small lumina and one large lumen are present in this field. × 200.

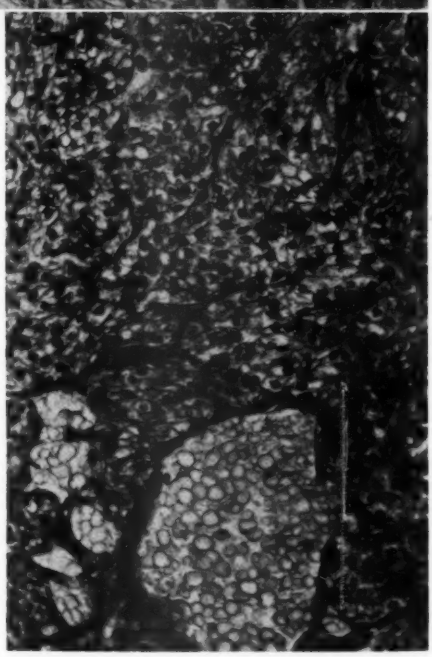
FIG. 10. There is beginning cyst formation due to degeneration of tumor cells. Some of the cells show mucoid degeneration. × 400.

FIG. 11. A small cyst is filled with degenerated, anuclear, clear cells. × 200.









Lever and Castleman

Clear Cell Myo-epithelioma

ANOMALIES OF MAJOR CEREBRAL ARTERIES ASSOCIATED WITH CONGENITAL MALFORMATIONS OF THE BRAIN

With Special Reference to the Pathogenesis of Anencephaly *

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Congenital anomalies of the central nervous system, including anencephaly, can be induced readily by means of anoxia,[1,2] irradiation with x-rays,[3] and by alterations in the thermal,[4,5] osmotic, or ionic environment of the developing embryo.[1,3] Furthermore, the nature of the induced malformation seems to depend more upon the stage of embryonic development at the time of the experiment than it does upon the means by which the injury is induced. These facts have suggested to Stockard and others[1,4] that the experimental anomalies probably result from an interference with fetal metabolism, with arrest or retardation of embryonic development in the areas of greatest metabolic activity.

To learn more about the pathogenesis of congenital malformations of the brain in human beings, detailed post-mortem examinations were performed on 14 anencephalic monsters. In light of the previous experimental findings and in view of the dependence of embryonic development upon an adequate supply of oxygen, particular attention was paid to the cerebral arterial system, and an original injection technic was devised for a more accurate study of the cerebral blood vessels. In addition, alterations were induced in the brains of developing chick embryos by occluding one or another of the major cerebral arteries, and these were studied in relation to the naturally occurring congenital anomalies of the brain.

Summary of Post-Mortem Findings in Fourteen Cases of Anencephaly

The cerebral malformations were characteristic in all 14 cases of anencephaly. The forebrain was regularly involved, while the midbrain, medulla, cerebellum, and cervical region of the spinal cord were spared or involved to a lesser degree, the findings conforming to those

* This investigation was supported by a research grant from the National Institute of Neurological Diseases and Blindness of the National Institutes of Health, Public Health Service.

Presented at the Forty-eighth Annual Meeting of the American Association of Pathologists and Bacteriologists, Cleveland, April 26, 1951.

Received for publication, February 1, 1952.

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previously described by others.[6,7] The calvarium was partially or totally absent in each case and the cerebrum was replaced by an irregular mass of hemorrhagic fibroglial tissue within which were occasional groups of poorly differentiated nerve cells and a number of cysts, often lined by ependyma and sometimes containing choroid. Everywhere in the fibroglial tissues there were numerous engorged, thin-walled blood vessels that varied markedly in size and shape. Histologic studies, to be described in detail later, disclosed that these vessels had but little muscular tissue within their media and therefore more closely resembled veins than arteries. Leukocytes were found in the cerebral tissues only in regions adjacent to areas of superficial ulceration.

Elsewhere the central and peripheral nervous systems showed no constant abnormalities on macroscopic and microscopic examination. Cranial nerve ganglia were regularly present in the base of the skull and from them nerve trunks extended in either direction, ending cephalad within the dense mesenchymal tissue. The ganglion cells were well formed, while the cranial nerves were composed principally of sheath cells, although in their peripheral portions they contained nerve fibers as well. The spinal cords did not differ appreciably from the normal, either in size, in number and distribution of nerve cells, or in quantity of white matter. The dorsal root ganglia were regularly present and the ganglion cells here were normal in appearance, as were those in the celiac and visceral plexuses of the small and large intestine and about the adrenal glands. The thoracolumbar sympathetic chains were readily identified grossly, and the ganglion cells appeared normal in histologic sections.

Two eyes were present in each of the 14 cases. They were normal in size and gross appearance, although proptosed because of shallowness of the orbits. Within them, the structures derived from facial ectoderm, namely, the lens and cornea, appeared normal in histologic preparations. The retina, a derivative of the diencephalon, was regularly formed of well defined rods and cones, an outer and inner nuclear layer, a ganglion cell layer with fewer than normal nerve cells, and a fiber layer of varied thickness that contained a few fibers as well as glial cells. A short segment of optic nerve frequently was present, extending posteriorly from the orbit to the region of the optic foramen. Within this, the glial and septal connective tissue predominated but nerve fibers were present also.

The anterior lobe of the pituitary body, unaccompanied by a posterior lobe, was identified at the base of the skull in the 4 cases in which this region was studied by serial section; this finding is in agreement

with the observations of others.[8,9] Marked adrenal hypoplasia was present regularly, while such features as harelip, cleft palate, spina bifida, hyperplasia of the thymus, hydronephrosis and hydroureter, and abnormalities of major visceral arteries were present also in some cases. These findings and other data relative to the 14 cases are summarized in Table I.

Anomalies of Major Cerebral Arteries in Five Cases of Anencephaly as Demonstrated by a Fusible-Metal Injection Technic

To study more accurately the blood supply of anomalous brains, a new technic was devised whereby the vessels are injected with a fusible metal; this hardens upon cooling and can be studied *in situ* by means of radiography or by examination of the casting which remains after digestion of the tissues with alkali.

The materials and methods in this study have been described in detail elsewhere.[10] In brief, they consist of the intra-arterial injection of cerrolow 117,* a eutectic alloy of bismuth, lead, indium, tin, and cadmium with a melting point of 117° F. Before injection, the cerebral vascular bed is perfused thoroughly with kerosene through a large cannula inserted in the thoracic aorta. The cadaver is then heated in a water bath at a temperature of 120° F. and the perfusion continued with warm kerosene. The warmed metal is then injected at a maintained pressure of 150 to 180 mm. of Hg; the cerebral arteries are thus filled with fused metal, which hardens when cooled. Because of its unusual density, the metal serves as an excellent contrast medium for stereoscopic x-ray studies; furthermore, the soft tissues can be dissolved away readily with a 20 per cent solution of potassium hydroxide, leaving rigid metallic castings of the arterial system.

The technic has been employed in a study of 5 cases of anencephaly with the following results.

Case 10

In case 10 the cephalic malformation was characterized by an absence of the left half of the calvarium and replacement of the left cerebral hemisphere by a soft cystic mass of hemorrhagic tissue. A large cleft extended from the left lateral margin of the mouth to the left orbit and the left eye was displaced deep within it (Figs. 1 and 2). In addition, a median cleft was present in the upper lip. Figure 3 shows the metallic casting of the vascular system in this case. Two medium-sized carotid arteries can be seen, their bifurcations forming internal and external carotid vessels. The right internal carotid artery

* Cerro de Pasco Copper Co., New York, N.Y.

TABLE I

Summary of Post-mortem Findings in Fourteen Cases of Anencephaly

Case no.	Birth Weight	Duration of life	Sex	Malformations of nervous system	Other lesions
	gm.				
1	1700	Born dead	M	Craniorhachischisis with cervical spina bifida, anencephaly, and cervical amyelia	Cleft palate; incomplete rotation of intestine; hypoplasia of adrenal glands (1.1 gm.*)
2	2040	Born dead	F	Cerebrum and cerebellum replaced by a 4 x 3.5 x 1 cm. mass of hemorrhagic tissue; calvarium absent	Hydroureter and hydronephrosis; hypoplasia of adrenal glands (0.5 gm.*)
3	2900	25 hours	M	Brain stem and small cystic mass of brain tissue present at base of skull; calvarium absent	Hypoplasia of adrenal glands (1.1 gm.*)
4	1140	Born dead	M	Forebrain replaced by hemorrhagic cystic mass; fronto-parietal defect of calvarium	Hypoplasia of adrenal glands (0.5 gm.*)
5	1150	Born dead	F	Cerebrum replaced by flattened mass of friable tissue; medulla poorly formed, calvarium absent	Hypoplasia of adrenal glands (0.5 gm.*)
6	2280	23 hours	M	Encephalon shrunken, cystic, and hemorrhagic; calvarium absent	Hyperplasia of thymus (20 gm.); hypoplasia of adrenal glands (1.4 gm.*)
7	950	Born dead	F	3 x 3 x 1 cm. mass of hemorrhagic tissue on floor of skull; medulla poorly formed; calvarium absent	Umbilical hernia; hypoplasia of adrenal glands (0.8 gm.*)
8	2800	26 hours	M	20 gm. of partially developed cerebral tissue with gyri, meninges, and well defined medulla; calvarium absent	Hypoplasia of adrenal glands (1.0 gm.*)
9	3270	6 hours	F	3 x 3 x 1 cm. mass of cerebral tissue attached to spinal cord; calvarium and posterior vertebral arches in cervical region absent	Cleft palate, harelip; hypoplasia of adrenal glands (1.0 gm.*)
10	2840	2 hours	F	Left cerebral hemisphere replaced by hemorrhagic cystic mass; calvarium absent over left cerebral area (Figs. 1, 2)	Median harelip and lateral facial fissure; hypoplasia of adrenal glands (1.1 gm.*)
11	3800	31 days	M	Forebrain replaced by 3 x 3 x 2 cm. mass of hemorrhagic tissue; fronto-parietal portion of calvarium absent (Fig. 4)	Hypoplasia of adrenal glands (0.8 gm.*)
12	2800	Born dead	F	Cerebrum, cerebellum, and brain stem replaced by flattened mass of hemorrhagic tissue; craniorhachischisis (Figs. 6, 7)	Right subclavian artery in retro-esophageal position; hypoplasia of adrenal glands (0.3 gm.*)
13	2150	Born dead	F	Similar to case 12	Hypoplasia of adrenal glands (0.8 gm.*)
14	2290	40 hours	M	Similar to case 12	Hyperplasia of thymus (22 gm.); hypoplasia of adrenal glands (0.4 gm.*); hydroureter and hydronephrosis

* Weights of both adrenal glands.

penetrated the base of the skull and was joined by a posterior communicating branch with the basilar artery, thus forming one half of a circle of Willis. A single slender artery arose from the point of union of the right internal carotid and posterior communicating arteries and extended through the right cerebral hemisphere in a course resembling that of the normal middle cerebral artery. The left internal carotid artery, in contrast to the right, did not unite with other arteries in the circle of Willis, but ended abruptly several millimeters distal to the point of its emergence from the base of the skull. There were no major arteries extending from it into the area of cerebral malformation.

Case 11

Case 11 was an infant who lived 31 days, manifesting regular respirations and a sucking reflex. The calvarium was absent, except in the lower occipital region, and the cerebral hemispheres were symmetrically replaced by friable hemorrhagic tissue (Fig. 4). The metallic casting of the arterial system (Fig. 5) showed the presence of two internal carotid arteries that joined in an inverted V formation a short distance from their points of entry into the cranium. From the site of union of these two vessels, a slender and tortuous artery extended posteriorly in the midline to unite with an extremely long and narrow basilar artery. A plexus of arteries arose from this midline vessel, but these were small in caliber, and in distribution they bore no resemblance to the usual pattern of anterior, middle, and posterior cerebral arteries.

Cases 12, 13, and 14

The cerebral and cranial deformities in cases 12, 13, and 14 were similar and were characterized by a craniorhachischisis that extended posteriorly to involve the upper cervical vertebrae and by the replacement of the cerebrum, cerebellum, midbrain, and brain stem by flattened masses of hemorrhagic tissues (Figs. 6 and 7).

Stereoscopic radiographs of the alloy-injected cadaver of case 12 showed two small internal carotid arteries that penetrated the base of the skull and gave origin to a number of slender vessels that angulated posteriorly, then upwards and anteriorly through the cerebral mass. Neither an anterior nor a posterior communicating vessel could be identified. The vertebral arteries arose from the subclavian vessels and arched posteriorly and then anteriorly and cephalad to approximately the level of the second cervical vertebra, while decreasing progressively in caliber. They ended independently. Thus, there was neither a basilar artery nor a visible union between the vertebral and internal carotid systems; there was no circle of Willis (Fig. 8). In

addition, the right subclavian artery was anomalous. It arose from the arch of the aorta in a near normal position but passed behind the esophagus and trachea before resuming a normal position again as the right brachial artery.

The metallic casting of the cerebral arterial bed in case 13 showed malformations almost identical with those of the previous case. The two internal carotid arteries were widely separated as they penetrated the base of the skull. There were no communicating arteries, but a number of irregular slender vessels arose from the carotid arteries and coursed through the cerebral mass in an anterior-posterior direction. As in the previous case, the vertebral arteries tapered and ended in the upper cervical region without uniting to form a basilar artery. Again, there was no communication between the carotid and vertebral circulation and no circle of Willis (Figs. 9 and 10).

The metallic casting of the arterial system in case 14 (Fig. 11) showed the two internal carotid arteries penetrating the base of the skull and then branching laterally through the cerebral mass. The ophthalmic arteries were large and arose from the internal carotid vessels at points within the sphenoid bone. The numerous intra-ocular and peri-orbital arteries were widely and regularly distributed. The vertebral arteries filled imperfectly with the alloy but again no circle of Willis was identifiable.

The regular association of abnormalities of the major cerebral arteries with cephalic deformities in anencephalic monsters raised the question whether a specific intrinsic structural alteration or morphologic lesion might be present within the walls of the deformed cerebral arteries. To investigate this possibility blocks of tissue were taken transversely through the internal carotid and vertebral arteries from the base of the skull and lower surfaces of the anomalous cerebrum and also from the upper cervical and medullary regions of the vertebrae and brain stem in 3 of the cases (nos. 7, 8, and 9). Histologic sections were made at different levels and stained with hematoxylin and eosin, by the Masson trichrome method, and by the Weigert stain for elastic tissue.

Sections of the intrasphenoidal segments of the internal carotid arteries and the cervical segments of the vertebral arteries showed the lumina of these vessels to be patent and the walls to be formed of a thin intima, a media containing a moderate quantity of muscle tissue, and a scant adventitia. No significant alterations were noted within the walls of these arteries; and specifically there was no evidence of arteritis or of arterial thrombi. Although numerous large and small

vascular channels were present in sections through the base of the anomalous brains and medullae, most of these vessels resembled veins or venous sinusoids, there being little or no muscular tissue within their media.

Malformations Induced in the Brains of Chick Embryos by Occluding Cerebral Arteries

The question remained open whether vascular anomalies such as those just described are responsible for the cerebral malformations or whether they are secondary to them. To gather more information on this point, one or another of the arteries in the brains of developing chick embryos was occluded by electrocauterization, and the resulting alterations in cerebral development were studied.

The apparatus for cauterization consisted of two 45 volt dry cell batteries placed in series with a milliampere meter and a governable resistance to deliver from 0 to 25 m.a. of current. The cathode or cauterizing electrode was a silver wire drawn to a diameter of approximately 0.25 mm., pointed sharply, and insulated except at the extreme tip with Ajax insulating material.* The anode or neutral electrode was a small rectangular silver plate bent sharply to fasten over the edge of the opened shell. All procedures were carried out under aseptic conditions on the stage of a stereoscopic, dissecting microscope in a room-incubator at a temperature of 98.6° F. White eggs with a better than 90 per cent fertility rate were used.

A window, 0.5 by 1 cm. across, was made in the shell immediately above the 6-day-old embryo and the sterilized neutral electrode was fastened securely on the window edge. The cauterizing electrode, supported by a burette stand and clamp with a universal swivel joint, was manipulated into the embryo adjacent to the carotid or major cerebral artery (Fig. 12). A current of from 8 to 10 m.a. was applied for periods of 5 to 15 seconds, as required to cause blanching of the vascular bed distal to the point of cauterization. To enhance thrombosis and to minimize hemorrhage the electrode was left in place with gentle pressure on the vessel for a period of several minutes. In a few instances, a 1 mm. segment of the tip of the electrode was severed and left embedded at the point of cauterization. The shell window was replaced and sealed with paraffin. Approximately 80 per cent of the embryos in which one carotid artery had been occluded died during the subsequent 5 days, bleeding from the site of cauterization being a frequent cause of death. In a control experiment to learn whether the

* A clear, air-drying varnish, obtained from Sherwin-Williams Co., Cleveland, Ohio.

alterations that followed occlusion of a cerebral artery had resulted from the impairment of the blood supply or had been produced directly by the trauma of cauterization, a number of chick embryos were treated like those of the experimental group, except that the tip of the cauterizing electrode was placed within the cervical or cephalic region but not in a position to cause thrombosis of a major cerebral artery.

After the eggs had been incubated for an additional 5 to 7 days, the shell windows were removed and the contents of the amniotic sacs were cultured in infusion broth. The living embryos of the experimental and control groups were placed directly into formaldehyde solution, and after fixation histologic sections were prepared and stained with hematoxylin and eosin and by the Masson trichrome method.

Pronounced malformations regularly followed occlusion of a carotid artery in the cervical region; these were not present in embryos of the control group. The cerebral hemisphere and the eye on the side of the occluded vessel were always retarded in development while the forebrain often remained as a large thin-walled cyst, the apical portion of which was formed by the markedly narrowed cerebral cortex (Figs. 13 and 14). This consisted of a thin layer of undifferentiated nerve cells not arranged in any definite pattern. The cells had uniform, small, hyperchromatic nuclei and little cytoplasm with no identifiable axonal processes. The underlying white matter contained few cells and abundant fibrillar tissue (Fig. 15). The degree of cellular differentiation was conspicuously less than that of the well formed cells with large nuclei, abundant cytoplasm, and axonal processes, which were present in corresponding areas of the brains of embryos of the control group (Fig. 16). It is noteworthy that the alterations were regularly bilateral, perhaps because in the 6-day-old chick embryo communication is already established between the internal carotid arteries, so that occlusion of one diminishes by one-fourth to one-third the blood supply to both cerebral hemispheres.

Occlusion of a cerebral artery at the base of the forebrain was followed by less pronounced alteration in the development of the cerebral hemispheres and no impairment in the development of the eye. When occlusion was accomplished proximal to the ophthalmic artery, however, the eye on that side was markedly retarded or arrested in development, as evidenced by a failure to increase in size and by a lack of cellular differentiation within the retina. Under these conditions, the retinal cells had uniform, small hyperchromatic nuclei; they did not differentiate into rods and cones, or form nuclear and ganglion cell layers, although their counterparts in the contralateral eyes and in

those of embryos of the control group regularly did so (Figs. 17 and 18).

Discussion

In the 14 cases of anencephaly here reported, the cerebral anomalies occurred regularly in the region normally supplied by the internal carotid arteries, while the adjoining parts—the eyes anteriorly and the medulla posteriorly—which receive their blood from the ophthalmic and vertebral arteries, respectively, were more normally formed. In addition, portions of the cranial nerves and their ganglia were well developed.

These findings make it plain that the anencephaly was not due to a defect in the initial formation of the encephalon, for this structure is normally laid down first in the development of the brain, while the optic cups and the anlagen of the cranial nerves form as appendages which bud out from it sometime during the third to fifth week of fetal life. Hence, it would be illogical to suppose that the encephalon could be initially malformed and still give rise to normal structures of the latter sorts. Furthermore, since the retinal cells are derived directly from neuroblasts of the diencephalon and since they are well differentiated and well preserved in the anencephalic monster, it may be inferred that the cerebral malformation is not due to an intrinsic defect of the neural ectodermal cells themselves. It would seem not unlikely that the retinal neuroblasts are unaffected in anencephaly because they migrate from the cranium and subsequently acquire a blood supply different from that of the cerebrum.

The presence of glial and mesenchymal tissues and the paucity of the more specialized neural elements in the malformed brains of anencephalic monsters provide additional evidence that impaired vascularity may be responsible for the malformation, for the vulnerability of neurons to anoxia is well known. By means of a special injection technic, anomalies of the major cerebral arteries were actually demonstrated in 5 of the cases of anencephaly here studied, and it seems especially noteworthy that the blood vessels involved were those that normally would have supplied the malformed regions. Microscopic studies failed, however, to disclose the precise nature of the vascular anomaly.

It is a dictum in embryology that slight curtailment of the normal blood supply to any part of the developing embryo will cause its growth to cease, while marked decreases will bring about local atrophy or malformation.[11] This was borne out in the present work, the artificial occlusion of a carotid artery in developing chick embryos regu-

larly leading to a marked retardation in the development of the brain and in the differentiation of the nerve cells. Indeed, the alterations so induced were similar in nature, although not so marked in degree, as those that characterize the lesions of anencephaly. Considered together, the findings indicate that anomalies of the major cerebral arteries developing sometime after the third to fifth week of fetal life may be an important factor in the pathogenesis of anencephaly in human beings.

Summary

Detailed morphologic studies of 14 cases of anencephaly showed that the cerebrum in each case was replaced by fibroglial tissue containing a few poorly differentiated nerve cells; by contrast, the cervical segment of the spinal cord, or the brain stem, or both, and the eyes, including the retinae, were in each instance well formed. The fact was noted that the malformed parts were those normally supplied by the internal carotid arteries, while the well formed parts were those normally supplied by the vertebral and ophthalmic arteries. Hence it seemed possible that anencephaly might result from some alteration in the blood vessels.

By means of injection of a fusible metal, anomalies of the major cerebral arteries were demonstrated in each of 5 cases of anencephaly studied in this way. The microscopic studies made thus far have failed, however, to disclose the nature of the vascular anomalies.

To learn whether induced vascular anomalies may lead to cerebral malformations, one or another of the cerebral arteries of developing 6-day-old chick embryos was occluded by electrocauterization; this procedure was regularly followed by a retardation of cerebral development and by alterations in the differentiation of the neuronal elements.

The possibility must be considered that vascular anomalies of the major cerebral arteries may be a factor in the pathogenesis of anencephaly in human beings.

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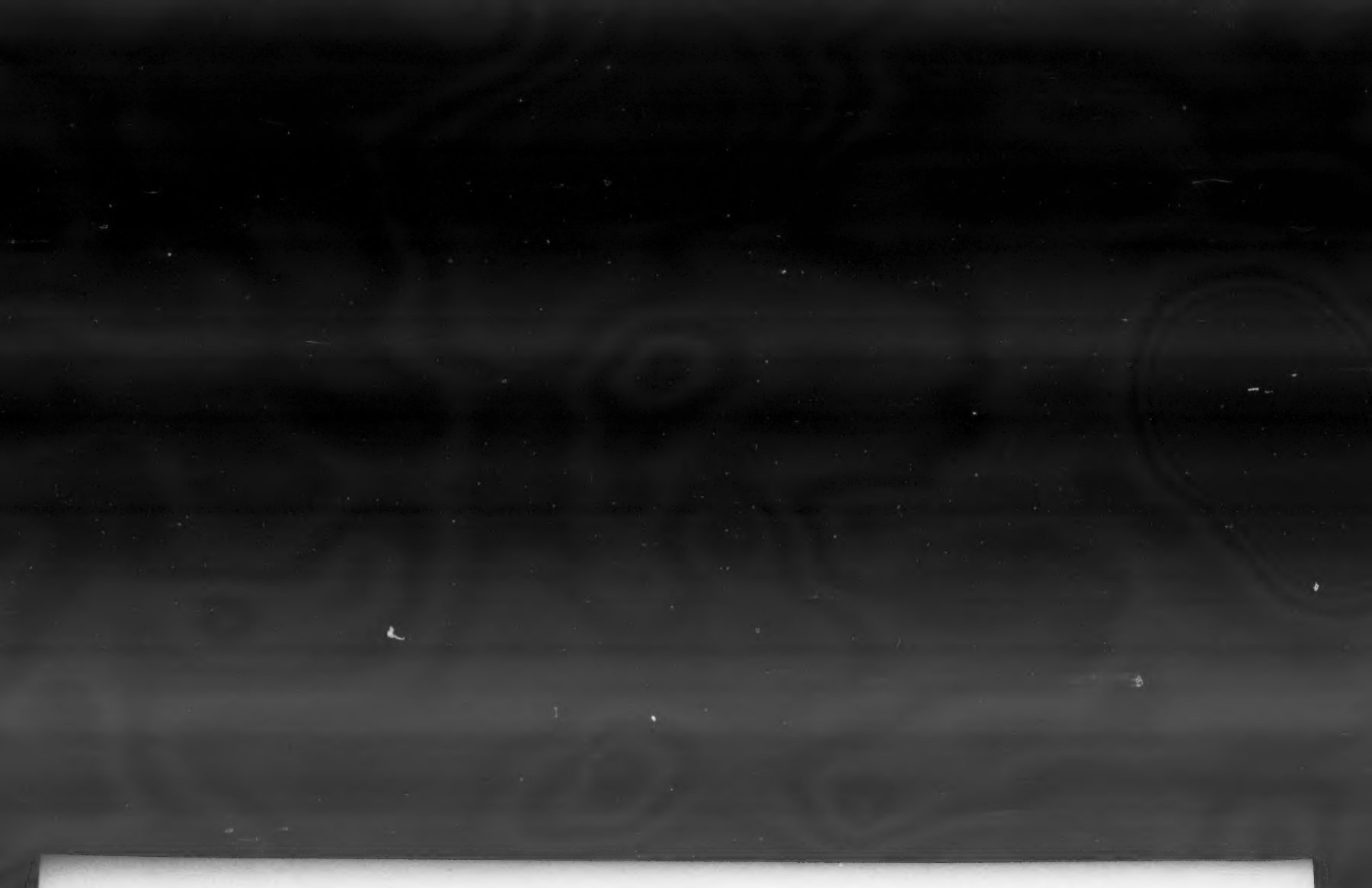
[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 100

FIGS. 1 and 2. Case 10. Anencephaly characterized by an absence of the left half of the calvarium and replacement of the left cerebral hemisphere by a large, cystic mass of hemorrhagic tissue.

FIG. 3. Metallic casting of the cerebral arterial system of the infant shown in Figures 1 and 2, prepared with cerrolow 117 according to a method described elsewhere in detail.[10] The two common carotid arteries (C.C.) bifurcate to form external and internal carotid arteries (E.C., I.C.). The right internal carotid artery unites by a posterior communicating vessel (P.C.) to the basilar artery (B.), which in turn communicates with the vertebrals (V.). A slender artery (M.C.) extends through the cerebral area in the distribution of the normal middle cerebral artery. The left internal carotid artery, slightly larger than its normal fellow, tapers abruptly and ends without joining the circle of Willis.



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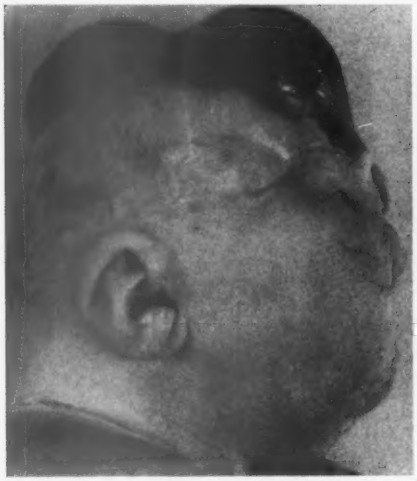

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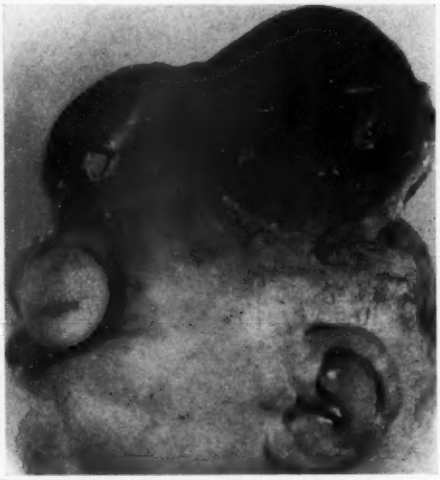

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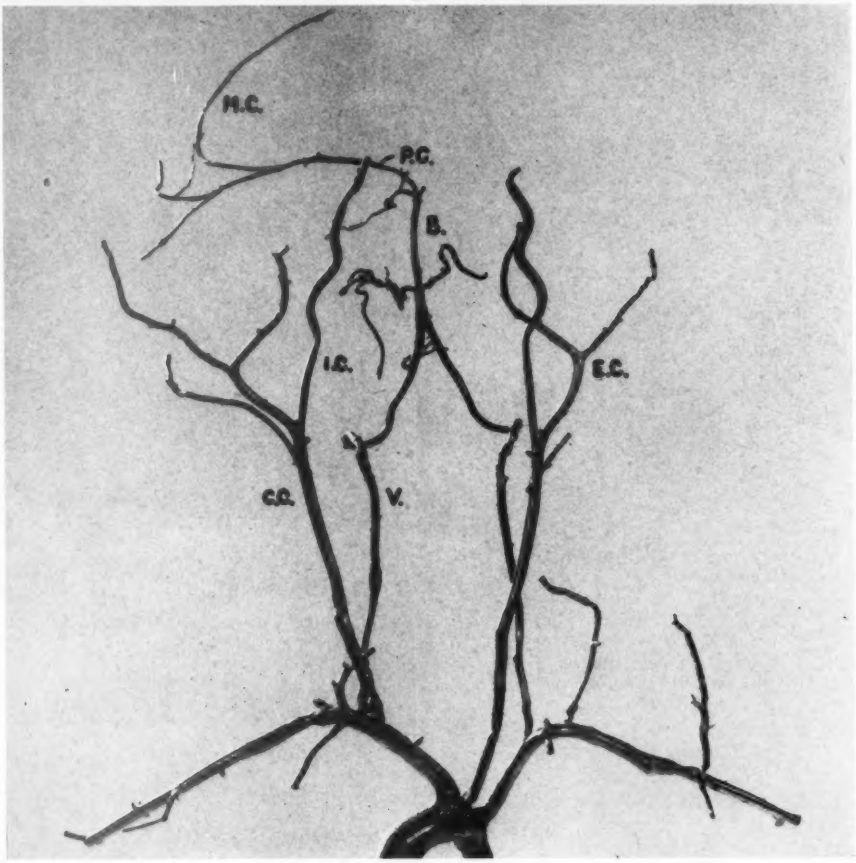
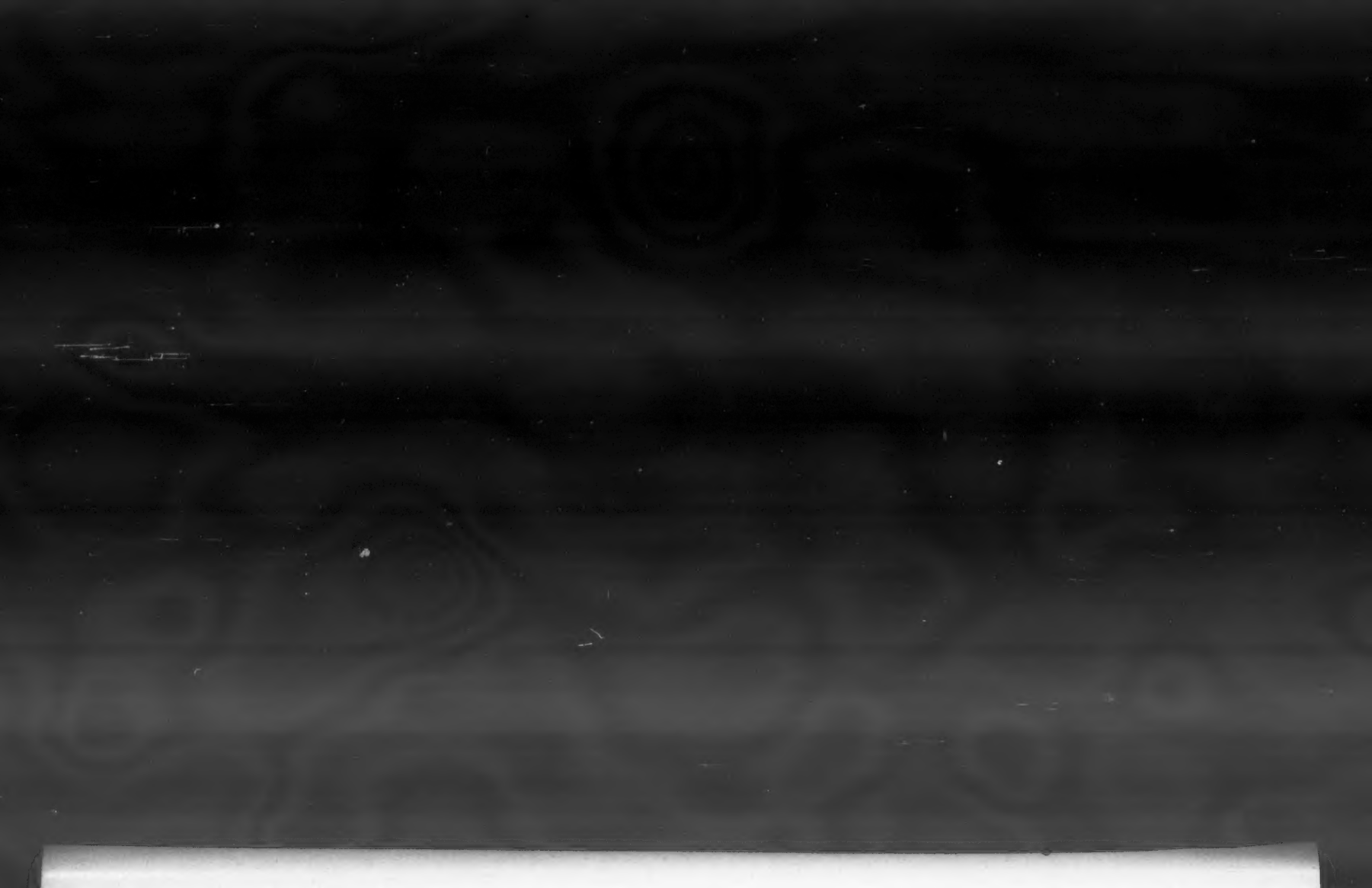


PLATE 101

FIG. 4. Case 11. A cerebral malformation characterized by an absence of the calvarium except in the lower occipital region and replacement of the cerebral hemispheres by a friable mass of hemorrhagic tissue.

FIG. 5. A metallic casting of the cerebral arteries of the anencephalic monster shown in Figure 4. The internal carotid arteries join in an inverted V formation and unite by a tortuous, midline vessel with an extremely long and slender basilar artery. A plexus of small, irregular arteries arises from the midline vessel. The ophthalmic arteries (O.) project anteriorly.



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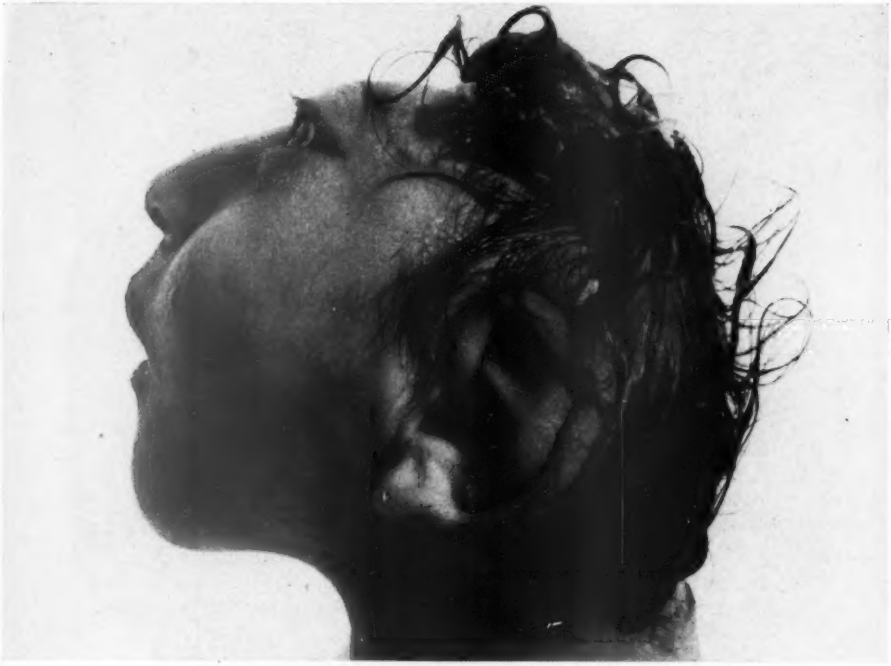

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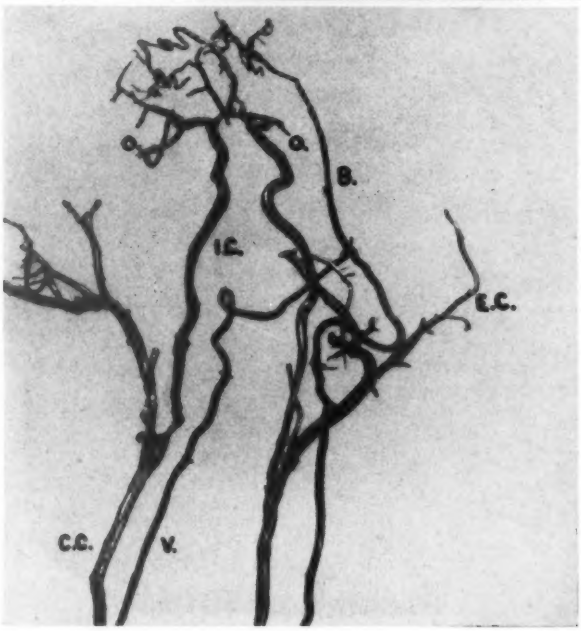


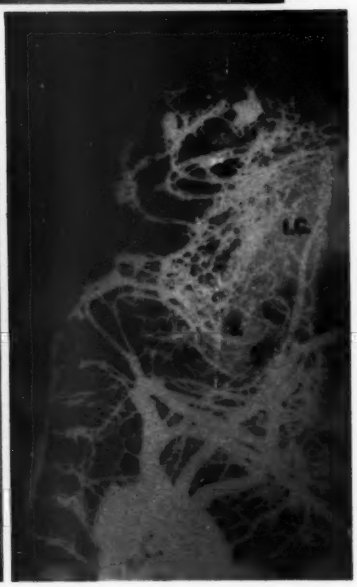
Vogel and McClenahan Pathogenesis of Anencephaly

PLATE 102

FIGS. 6 and 7. Case 12. A cephalic malformation characterized by craniorhachischisis associated with replacement of the cerebrum, cerebellum, and brain stem by a flattened mass of hemorrhagic tissue.

FIG. 8. Case 12. A roentgenogram of the alloy-injected cadaver. Irregular slender arteries arise from the internal carotid vessels and course first posteriorly, then superiorly and anteriorly through the cerebral mass. The vertebral arteries end independently in the cervical region.

6



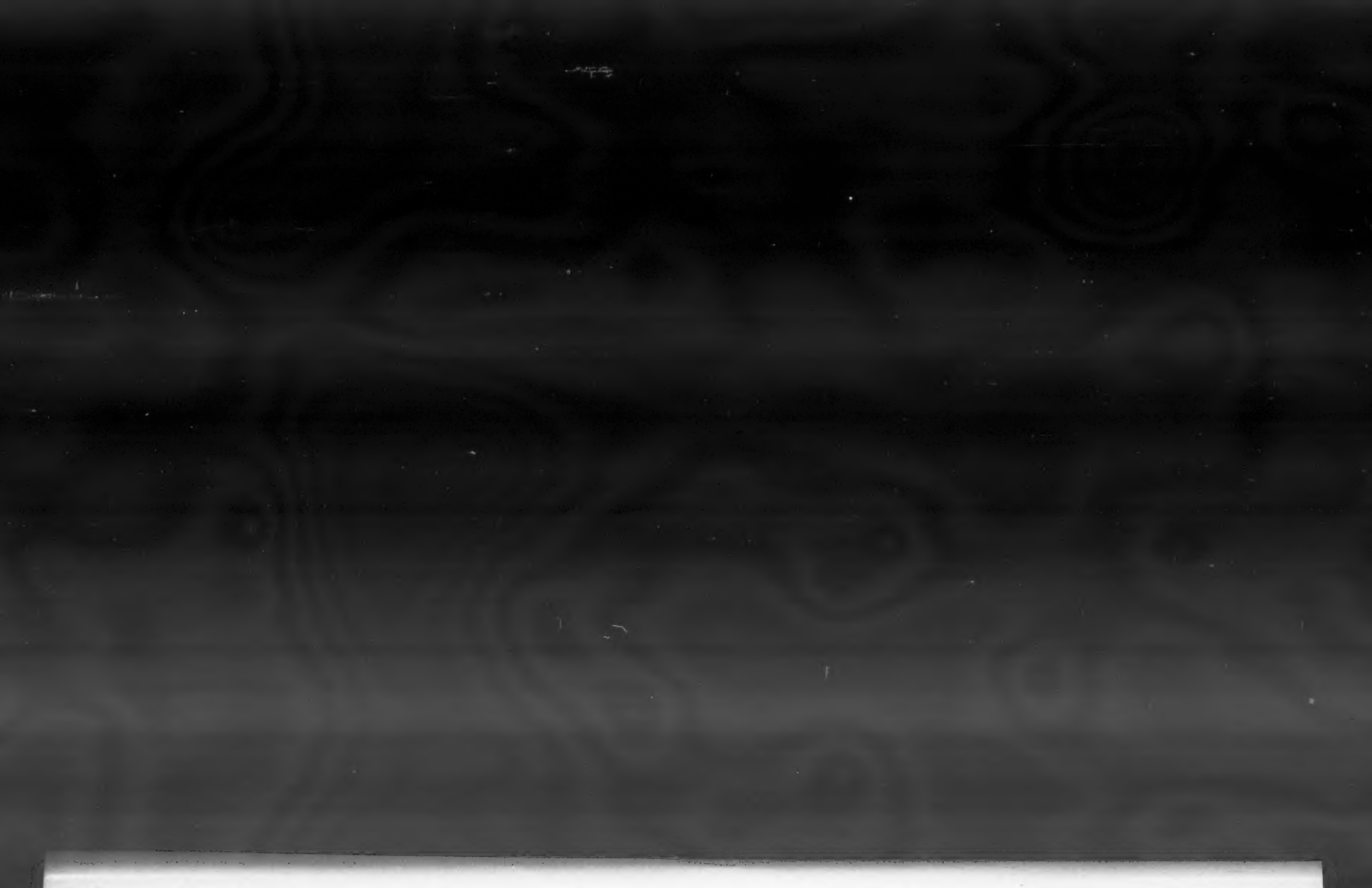
8

Vogel and McClenahan Pathogenesis of Anencephaly

PLATE 103

FIGS. 9 and 10. Case 13. Metallic casting of the cerebral vascular system of an anencephalic monster with a cephalic deformity similar to that pictured in Figures 6 and 7. The internal carotid arteries are widely separated and there are no communicating vessels. Slender arteries arise from them to supply the cerebral mass. The vertebral arteries do not unite to form a basilar artery, nor do they anastomose with the carotid arteries to form a circle of Willis.

FIG. 11. Case 14. Metallic casting of the cerebral vascular system, viewed from above with the sphenoid bone in place, from an anencephalic monster with craniorhachischisis and marked deformity of the cerebrum, cerebellum, and brain stem. The cerebral mass is supplied by a plexus of laterally directed vessels that arise from the internal carotid arteries. Large ophthalmic arteries arise from the intrasphenoid segments of the internal carotid arteries and numerous intra-ocular (I.O.) and peri-orbital (P.O.) vessels are well shown. Neither communicating arteries nor circle of Willis were to be found, and the vertebral arteries filled poorly with the alloy.



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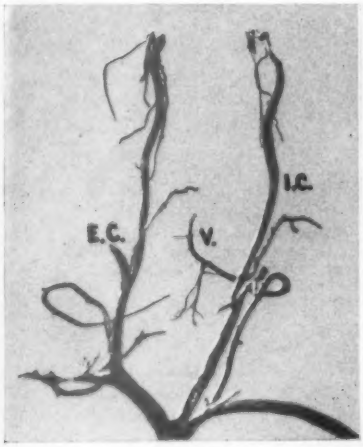

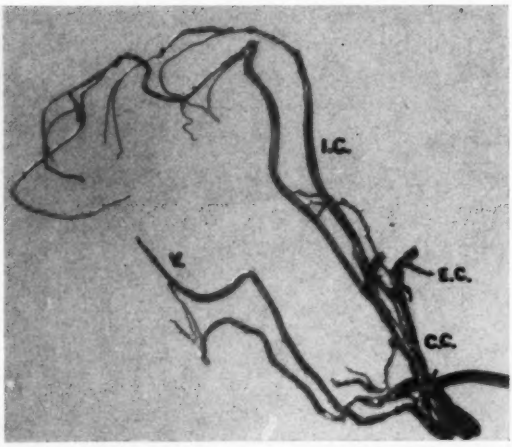

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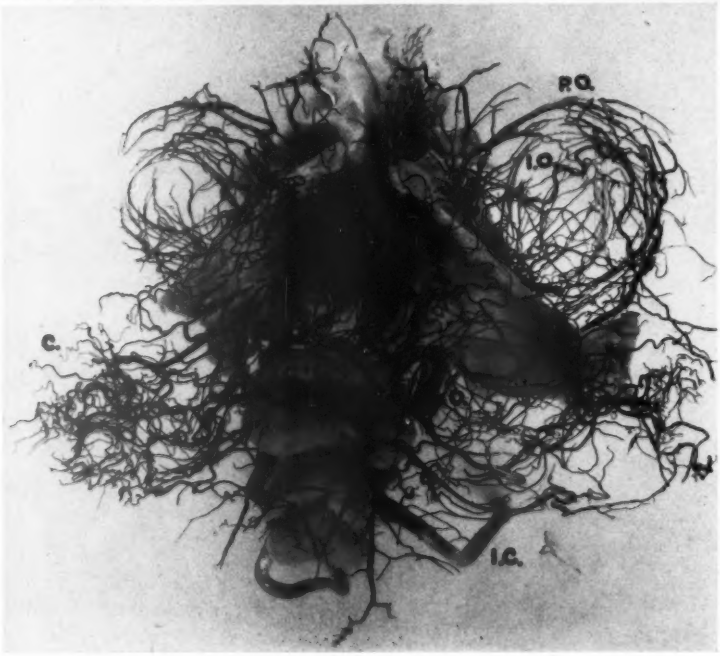


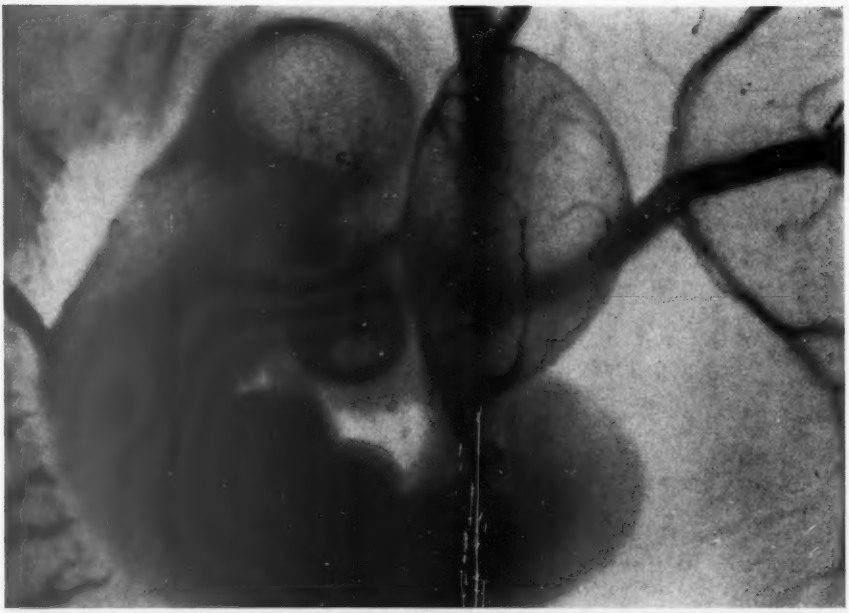
Vogel and McClenahan Pathogenesis of Anencephaly

PLATE 104

FIG. 12. The sites are shown where the common carotid artery (C.C.) and the cerebral arteries (C.) of a 6-day-old chick embryo were later occluded by electrocauterization.

FIGS. 13 and 14. A 12-day-old chick embryo. The right internal carotid artery was occluded by electrocauterization on the sixth day of incubation. The cerebral hemispheres are large, thin-walled cysts. The right eye measures 3 mm. across and is comparable in size to that of the 6-day-old embryo; the left eye has a diameter of 8.5 mm., and is normal in size for a 12-day-old embryo.

12



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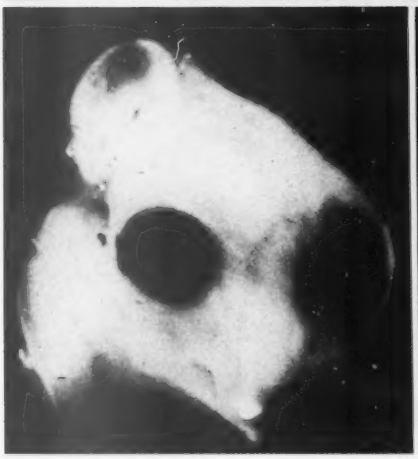

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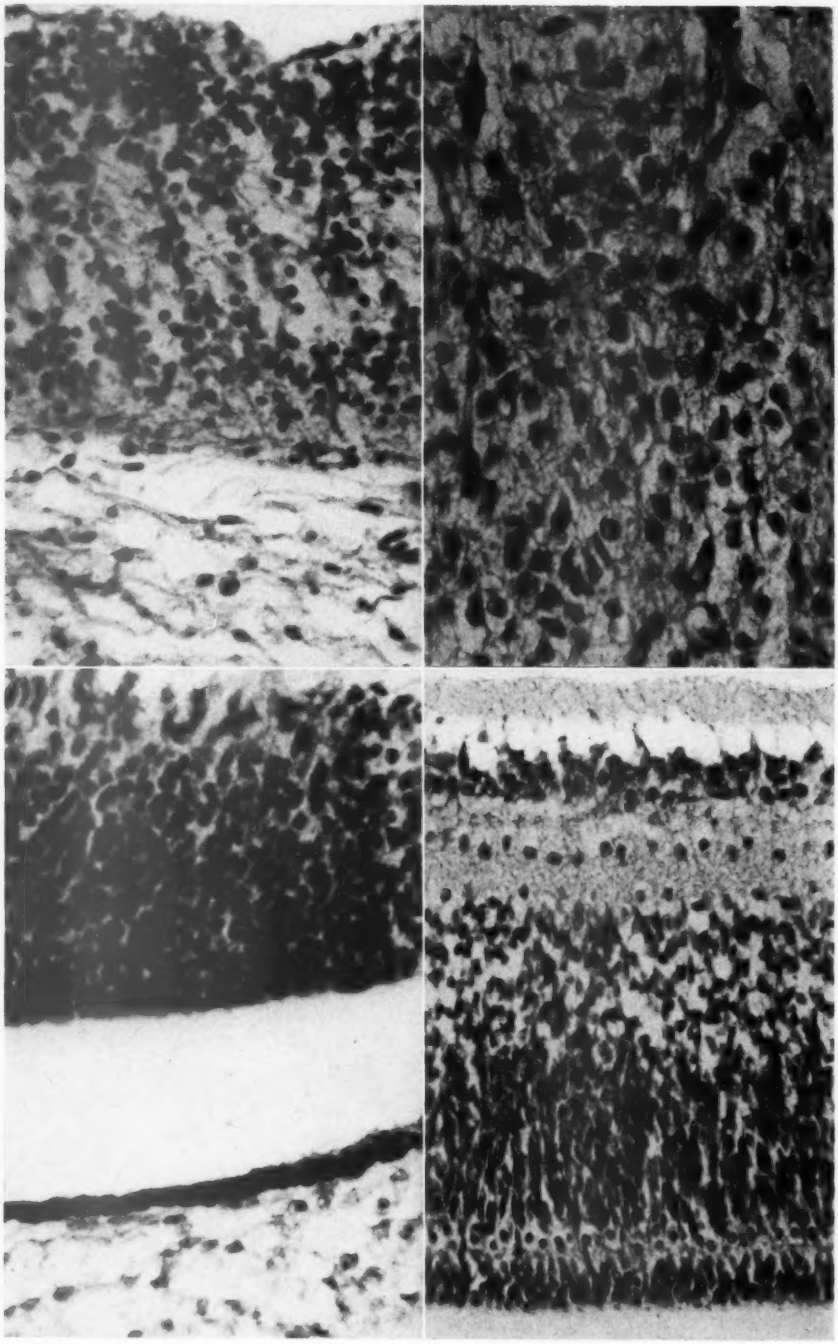
PLATE 105

FIG. 15. The cerebral cortex of a 12-day-old chick embryo following occlusion of the right carotid artery on the sixth day of incubation. The cortex is greatly narrowed and the cortical cells are undifferentiated, with uniform small hyperchromatic nuclei, very little cytoplasm, and no axonal processes. Hematoxylin and eosin stain. × 500.

FIG. 16. The outer layer of the cerebral cortex of a 12-day-old chick embryo of the control group. The nerve cells are well differentiated and have large nuclei with fine chromatin material and nucleoli and abundant cytoplasm with axonal processes. Hematoxylin and eosin stain. × 500.

FIG. 17. The retina of the right eye of a 12-day-old chick embryo 6 days after the occlusion of the right common carotid artery in the cervical region. The retinal cells have uniform, small, hyperchromatic nuclei and are not differentiated into rods and cones, nuclear or ganglion cell layers. The retina was detached. Hematoxylin and eosin stain. × 500.

FIG. 18. The retina of the left eye of the 12-day-old chick embryo from which Figure 17 was made. The retinal cells, in contrast to those of the right eye, are well differentiated into rods and cones, nuclear, and ganglion cells layers. Hematoxylin and eosin stain. × 500.



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17 18

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SHOCK DISEASE IN CAPTIVE WILD MAMMALS *

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Fatal convulsive seizures are known to become epidemic among certain species of free wild animals when their populations increase to peak levels.[1-4] In the individual animal the onset of the seizure follows the development of hypoglycemia; hence the term shock disease.[1,2] The depletion of carbohydrate reserves under these circumstances has not yet been explained fully. However, Christian[5] has suggested that adrenopituitary exhaustion, caused by the intrinsic stress of over-population, may be the basic cause.

In captive wild animals shock disease has, in our experience, developed under different circumstances. Nevertheless, the evidence suggests that its occurrence in these animals may be attributed to adrenopituitary exhaustion. It is hoped that a report of this material may contribute to an understanding of unusual susceptibility to stress.

MATERIAL

Fourteen examples of fatal shock disease will be described in this report. These animals were members of four families of the order Carnivora: the family Mustelidae was represented by 5 otters (*Lutra canadensis*) and 3 minks (*Mustela vison*); the family Felidae by a caracal (*Felis caracal*), a serval (*F. serval*), a European lynx (*F. lynx*), and a cheetah (*Acinonyx jubatus*); the family Procyonidae by a raccoon (*Procyon lotor*); and the family Canidae by an Arctic fox (*Alopex lagopus*). These animals had been captive for periods that ranged from 1 month to over 6 years. During this time each animal was seen at least twice daily, but evidence of illness was not observed.

In addition, 3 other animals have provided pertinent material. These are: (1) a raccoon-like dog (*Canis procyonoides*) killed for study because of progressive weight loss, and found to have recurrent focal necrosis of the pituitary body; (2) a young adult male raccoon (*Procyon lotor*) killed for study after having been isolated for 3 months in a small cage on an ample food supply; and (3) an adolescent male monkey (*Macaca radiata*) that died 5 days after severe injury. This monkey had been a member of a highly active family group.

These animals were dissected within a few hours after death. All

* Received for publication, February 23, 1952.

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tissues removed for histologic study were fixed in Bouin's solution, embedded in paraffin, and stained by routine methods.

Observations

Circumstances Under Which Shock Disease Developed

Ten animals of this series (4 otters, 3 minks, the European lynx, the cheetah, and the serval) died within a few hours after having been transferred to new exhibition sites. With the exception of the cheetah and the serval, these transfers were simply a matter of routine management. The cheetah and the serval, however, were moved because they had become increasingly excited by nearby building repairs and had failed to eat for 2 days. These 10 animals were under observation at the time of death and are known to have died during convulsive seizures.

Death of the caracal followed a brief fight with cagemates, although its injuries were limited to superficial scratches. Its death occurred during the night and was not witnessed. The Arctic fox also was found to have died during the evening hours, after having shown moderate excitement over repairs to adjacent cages. One otter and the raccoon were found dead in their accustomed cages. Neither of them had been disturbed, nor had they shown any signs of illness. Each of these animals had developed an abscess in the subcutis of the neck; otherwise evidence of disease was not recognized.

Post-Mortem Examinations

These 14 animals were mature specimens with well developed pelage and abundant, even excessive, firm white fat in all of the usual depots. In every animal the heart and aorta appeared smaller than normal, and in every animal all four chambers of the heart were dilated. The epicardial and endocardial surfaces were the sites of small, irregularly scattered hemorrhages. All organs, including the brain and its membranes, were intensely congested. The livers were moderately reduced in size and dark. The thyroid glands were within normal limits of size for animals on an adequate iodine intake, and were firm and dark red-brown.

Lymphoid tissues in every animal were hyperplastic, and their thymuses were large, compact, fleshy masses that contained numerous small hemorrhages. In the otters, for example, the weights of the thymuses ranged from 6 to 26 gm.; the bodies, from 7 to 11 kg. The thymus of adult otters should have undergone involution and weigh less than 1 gm. Lymph nodes were firm and often congested. The

spleens were firm, and on the sectioned surfaces numerous lymphoid nodules were easily visible.

The adrenal glands of all animals were reduced in size. For example, in the otters (the weights of which have been given) the combined weights of the adrenal glands ranged from 120 to 450 mg. In the caracal (which weighed about 8 kg.) the combined weight of the adrenal glands was about 800 mg. Thus, in these animals, the ratio of adrenal to body weight ranged from 2 to 10 mg. per 100 gm. of body weight, approximately. These values are about one-third the ratio of adrenal to body weight of free wild animals of the same families.[6] Adrenal atrophy of this degree is not unusual in captive wild animals, and can occur within a few months if activity be inhibited. Thus, in a young adult male raccoon, weighing 6.5 kg., that was kept for 3 months in a small cage with abundant food, the adrenal glands weighed 800 mg., or about 12 mg. per 100 gm. of body weight; this corresponds to the values for animals that died of shock disease. However, the adrenal glands do not invariably undergo atrophy in captive wild animals. For example, the young monkey that died 5 days after injury weighed 1.4 kg., and its adrenal glands weighed 1.6 gm., or over 100 mg. per 100 gm. of body weight. This animal, however, probably lost some weight during the time between injury and death, for it did not voluntarily take fluids during the last 2 days. But even if its body weight had been reduced by one-half (which is unlikely), the relative weight of its adrenal glands still would correspond to that of free wild animals.

Histologic Study

Adrenal Gland. In the otters, the minks, and the Arctic fox, the zona glomerulosa formed 20 to 25 per cent of the adrenal cortex. In other animals of this series this zone was less conspicuous, but still formed 10 to 15 per cent of the cortex (Figs. 1, 2, and 3). This high ratio is about three times that found in adult free wild animals of these families, or in captive wild animals that are members of active groups (Fig. 6). The prominence of the zona glomerulosa in shock disease of animals is attributed to atrophy of the other zones of the adrenal cortex. In the zona fasciculata atrophy was evidenced only by the relative length of the cell column, which was reduced by at least one-half, but in the zona reticularis atrophy was much more distinct. Often this zone was no more than ten small cells in width, and the nuclei of these stained solidly. The stroma of the reticularis was more conspicuous, as if it had coalesced into coarser strands as the glandular cells disappeared (Figs. 2, 3, 4, 8, and 9). The degree of atrophy of the adrenal glands of the otters, the minks, and the Arctic fox approxi-

mated that found in the raccoon-like dog, in which adrenal atrophy was attributed to necrosis of the pituitary body (Figs. 4 and 10).

In the remaining 4 animals of this series, namely, the caracal, the lynx, the cheetah, and the raccoon, the degree of atrophy was less striking. The appearance of their adrenal glands corresponded more closely to that of the raccoon (mentioned previously) which was killed for study after 3 months in a small cage on ample food allowance (Figs. 5 and 11). The microscopic appearance of the adrenal cortex of these animals contrasted sharply with that of the young monkey that had been a member of a highly active family group (Figs. 6 and 12). It died 5 days after having been isolated because of the injury that caused its death. As was to be expected, the cells of its adrenal cortex no longer contained lipids. Nevertheless, these cells were quite as large as those of the adrenal gland of the raccoon killed for study, in which lipids were present abundantly. In particular, the zona reticularis of the monkey's adrenal gland was made up of well defined cords of relatively large cells.

The apparent quantity of lipids contained in the cells of the zona fasciculata of the animals that died of shock disease usually decreased with the duration of stress. When death had occurred within a short time after the application of stress, the cytoplasm of these cells contained many small vacuoles and their nuclei were rather large and stained uniformly (Figs. 7 and 8). Reduction in the number of cytoplasmic vacuoles in the cells of the middle zone was most pronounced when stress had continued for more than 24 hours (Figs. 3 and 9). However, in the cheetah, about half of the cells of the zona fasciculata contained vacuoles after 2 days of stress.

The medullary cells of these adrenal glands presented highly uniform appearances. The cytoplasm was raggedly vacuolated or irregularly shrunken and cell membranes were disrupted. The characteristic basophilic granules of normal cells were almost completely absent.

Lymph Nodes. In the animals that died within an hour following stress the cortical tissue of the lymph nodes was composed of closely packed, large primary follicles. Usually these contained only mature lymphocytes. The so-called germinal centers were inactive or absent. The medullary cords were densely packed with mature plasma cells. The sinuses of the nodes contained many free lymphocytes and smaller numbers of plasma cells, and were moderately dilated. All blood vessels within the nodes also were dilated. As the time between stress and death increased, there seemed to be a moderate progressive reduction of mature lymphocytes in the primary follicles. Edema of the

medullary cords increased, and there was some reduction in the numbers of plasma cells. At this time all cells had disappeared from the sinuses. Also, as the time between stress and death increased, the germinal centers of the lymph nodes became more prominent, and mononuclear phagocytes and active phagocytosis became increasingly evident.

Thymus. The changes in the thymus closely paralleled those of the lymph nodes.

Spleen. The malpighian follicles of the spleen were definitely enlarged and composed of mature lymphocytes in the animals that died within a short time after stress. Subsequent changes corresponded to those in the lymph nodes. The spleens of all of the animals showed proliferation of the macrophage system (reticulo-endothelium). This hyperplasia was especially pronounced in the animals that survived for the longer periods and, as survival periods lengthened, phagocytosis of erythrocytes became increasingly more marked.

Liver. The liver cells were smaller than normal, and stained densely with eosin, regardless of the time between stress and death. The cytoplasm had lost completely the usual lacy appearance and basophilic granulation characteristic of the livers of well nourished animals. The nuclei varied widely; many were small and pyknotic; others were large and vesicular. The sinusoids and veins were distended, and the Kupffer cells usually contained phagocytized erythrocytes.

Pancreas. Sections from the pancreas were taken from only 2 of the animals: from an otter that died within 2 hours after the initial stress, and from the cheetah that had been under more or less continuous stress for 2 days before succumbing. In both, the secretory granules and the basophilia of the acinar cells were reduced and, in small well circumscribed foci, these cells had undergone necrosis without an associated inflammatory reaction. The islet cells appeared unchanged.

Thyroid Gland. When death occurred within a short time after stress, the thyroid glands were entirely normal for animals on an adequate iodine intake. The follicular epithelium was low to medium cuboidal, and the follicles usually contained a moderate amount of colloid of medium density and staining. Intracellular colloid droplets were small and located chiefly in the apical portions of the cells. As the interval between stress and death increased, however, there was a corresponding increase in the apparent activity of this gland. The follicular epithelium changed from cuboidal to columnar and became enfolded in many follicles. The nuclei of the follicular epithelium became larger and more vesicular. Colloid within the follicles largely

disappeared and that which remained, stained faintly. Intracellular colloid was increased, and located usually in the basal and mid-portions of the cell.

Discussion

These examples of shock disease were all young, adult, well nourished animals. At post-mortem examination they presented a uniform appearance. The circumstances under which death occurred also may be said to have been uniform. This statement is not intended to imply that, quantitatively, the fatal stimuli were equally severe. Nevertheless, it seems reasonable to suggest that these animals succumbed to stimuli that, under native environmental conditions, would have been trivial.

Under natural conditions the animals of this series are known to be very active, aggressive, and wary, and to range widely in search of food. The home range of the otter, for example, has been estimated to extend over as much as 100 miles of shore line, or 50 miles of stream,[7,8] and there is reason to believe that the other animals of this series are equally capable of sustained activity. Activity for carnivores of these types is much reduced in a zoological garden. Conflict with other animals of the same or a different species is rare. Food is abundant and supplied at regular intervals. The animals become well nourished, if not exceedingly fat, and their adrenal glands undergo partial atrophy. Exactly the same change has been found to occur in the adrenal glands of captive wild Norway rats.[9,10]

The manner in which these animals died and the changes found at necropsy can best be attributed to a reduced output of hormones from the adrenal cortex and the pituitary body. For example, 10 animals of this series were under observation at the time of death. Each of these died during a convulsive seizure and rigor mortis developed rapidly. The terminal convulsive seizure, the rapid onset of rigor mortis, and the apparent removal of glycogen from their livers, as well as from the livers of the 4 other animals in this series, support the opinion that these deaths were due to hypoglycemia.[1,2,11]

From the histologic changes found in the tissues the following explanation for the development of hypoglycemia is suggested. The stimuli to which the animals were subjected caused a rapid and continued release of epinephrine. The depleted state of the cells of the adrenal medulla indicates that this was so. The release of epinephrine may be presumed to have exhausted the stores of glycogen in the livers. At the same time glyconeogenesis failed to keep pace with the demand. In some instances this failure may be attributed to exhaustion of the adrenal cortex, but in a majority the cortex was not completely de-

pleted of lipids. In these instances it may be suggested that the output of ACTH was inadequate.[12-14]

Reduced activity of the pituitary gland and of the adrenal cortex seems to be a reasonable assumption in this series of animals. The secretion of ACTH and of 11-oxycorticosteroids is known to be interdependent. Inactivity and lack of stress in a zoological garden would be expected to reduce the production of ACTH and allow more or less atrophy of the adrenal cortex.

Adjustment of the pituitary and adrenal glands to reduced levels of activity would be entirely adequate for the ordinarily quiet life of a wild animal in captivity. Nevertheless, its automatic nervous system remains geared for violent activity; hence a reduction in the output of pituitary and adrenal cortical hormones becomes a distinct liability. It is suggested that this reduction can be prevented by careful control of food allowances. Reduced food will prevent obesity and stimulate greater activity, thus pituitary and adrenal function will continue at a more nearly normal level and the animals will be better prepared to withstand unusual stress.

In free wild animals the development of shock disease cannot be assumed to depend upon partial atrophy of the adrenal glands or reduced activity of the pituitary body. Instead, it has occurred when animal populations increased to peak levels, and competition and intraspecific conflicts were multiplied rapidly. Under these conditions adrenal secretions become inadequate and hypoglycemia results.[1,2,5] Shock disease, therefore, corresponds to the exhaustion phase of Selye's syndrome of general adaptation.[14]

Summary

Fourteen instances of death of captive wild animals from shock disease are reported. The animals, carnivores of small to medium size, are naturally adapted to lives of sustained and often violent activity. All of them died after being subjected to relatively minor stress, such as being transferred to new quarters or being disturbed by repairs to adjacent cages. Survival time after stress varied from a few hours to 2 days. Ten of the animals were under observation at the time of death, and are known to have died during a convulsive seizure. At necropsy all animals of the group were well nourished, if not exceedingly fat. In all of them lymphoid tissues were hyperplastic, the adrenal glands and the hearts were small, the livers were reduced in size, and dark. Histologic examination indicated that the glycogen stores of the liver were exhausted, and that the middle and inner zones of the adrenal cortex were hypoplastic. This evidence suggests that

the immediate cause of death was hypoglycemia. Hypoglycemia, in these animals, reflected their inability to respond normally to sustained stimulation because their adrenal cortices and, in some instances, their pituitary bodies had undergone partial atrophy through inactivity. Thus in them, shock disease resulted from an adjustment to inactivity, whereas in free wild animals the condition develops when environmental demands exceed maximum native capacities.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 106

FIG. 1. Adrenal cortex and medulla, American otter (*Lutra canadensis*), fatal shock disease after 31 months in captivity. The zona glomerulosa is more than one-half the width of the zona fasciculata, and the zona reticularis is indistinct. × 50.

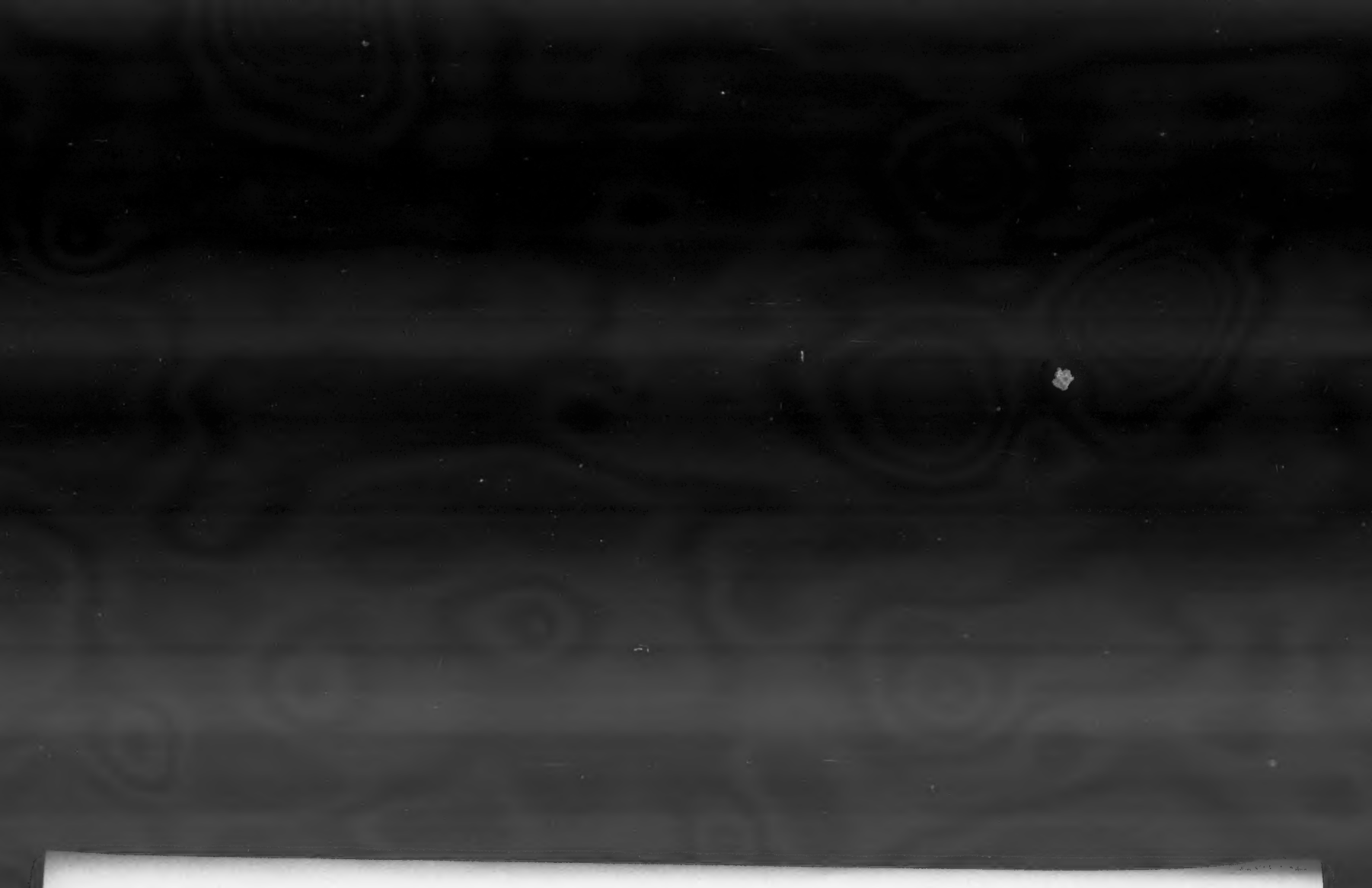
FIG. 2. Adrenal cortex, American otter (*L. canadensis*), fatal shock disease after 73 months in captivity. Atrophy of the zonae fasciculata and reticularis has not progressed so far as in the animal shown in Figure 1, but nevertheless the adrenal glands were less than half normal weight. × 50.

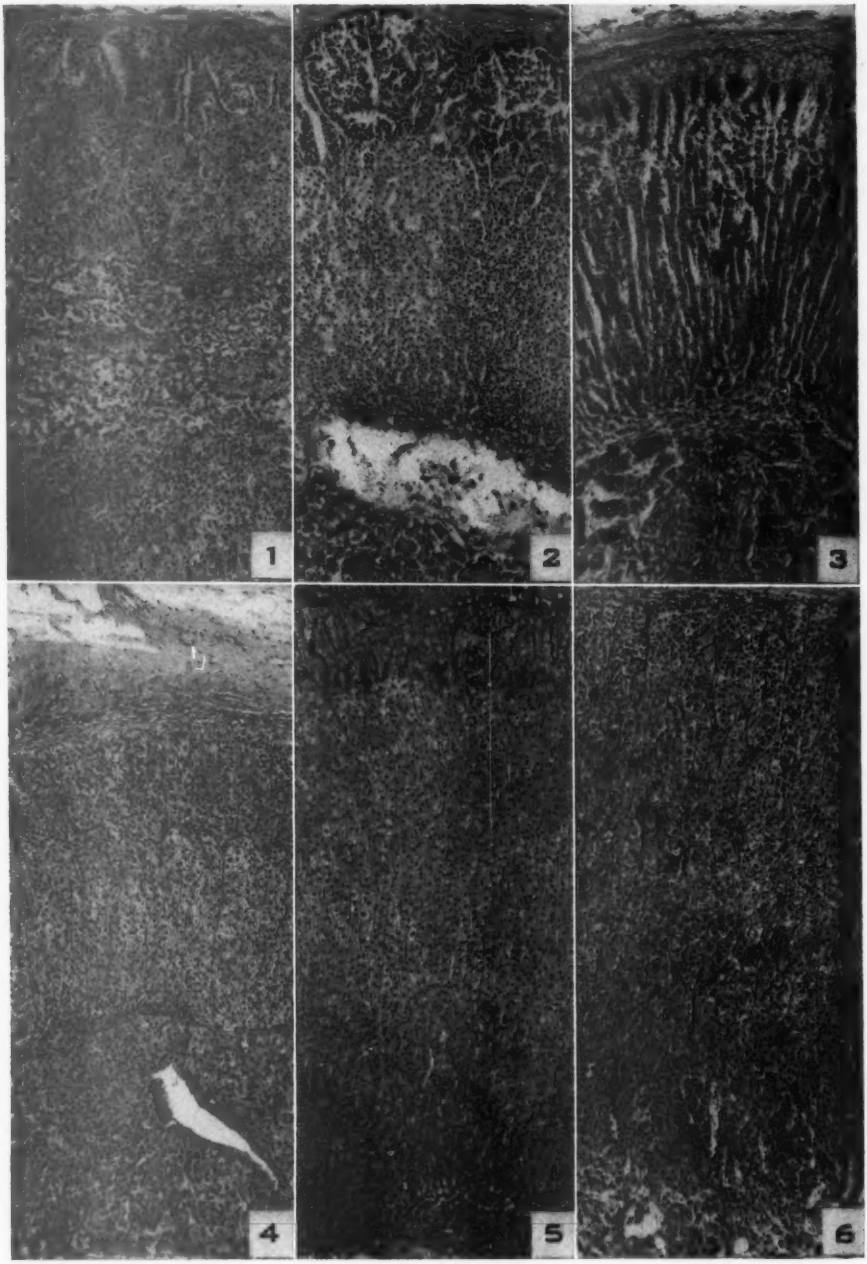
FIG. 3. Adrenal cortex, Arctic fox (*Alopex lagopus*), fatal shock disease after 18 months in captivity. The zona glomerulosa was conspicuous and, although the zona fasciculata was relatively wide compared to those in Figures 1 and 2, the zona reticularis consisted of only a few cells in a condensed stroma. Note also that in this adrenal gland lipids apparently had been exhausted. This was not true of the adrenal glands shown in Figures 1 and 2. × 50.

FIG. 4. Adrenal cortex and medulla, raccoon-like dog (*Canis procyonoides*), killed after 15 months in captivity, because of extreme weakness and weight loss, associated with pituitary necrosis. Atrophy of the adrenal gland in this animal was no greater than that shown in Figures 1, 2, and 3. × 50.

FIG. 5. Adrenal cortex, raccoon (*Procyon lotor*), killed after 3 months of inactivity and over-feeding. The zona glomerulosa was prominent, and the cells of the inner zona fasciculata and of the zona reticularis were reduced in size, and arranged in irregular cords. (*cf.* with Fig. 6.) × 50.

FIG. 6. Adrenal cortex, bonnet monkey (*Macaca radiata*), adolescent male, age 17 months, a member of a highly active group; death followed severe injury. The zona glomerulosa was inconspicuous, the zonae fasciculata and reticularis were well defined, and, although the cells of these zones were exhausted of lipids, they were as large or larger than the corresponding cells of the raccoon's adrenal glands (see also Figs. 10, 11, and 12). × 50.





Christian and Ratcliffe Shock in Captive Wild Mammals

PLATE 107

FIG. 7. Zonae fasciculata and reticularis and outer cells of medulla of adrenal gland shown in Figure 1. × 250.

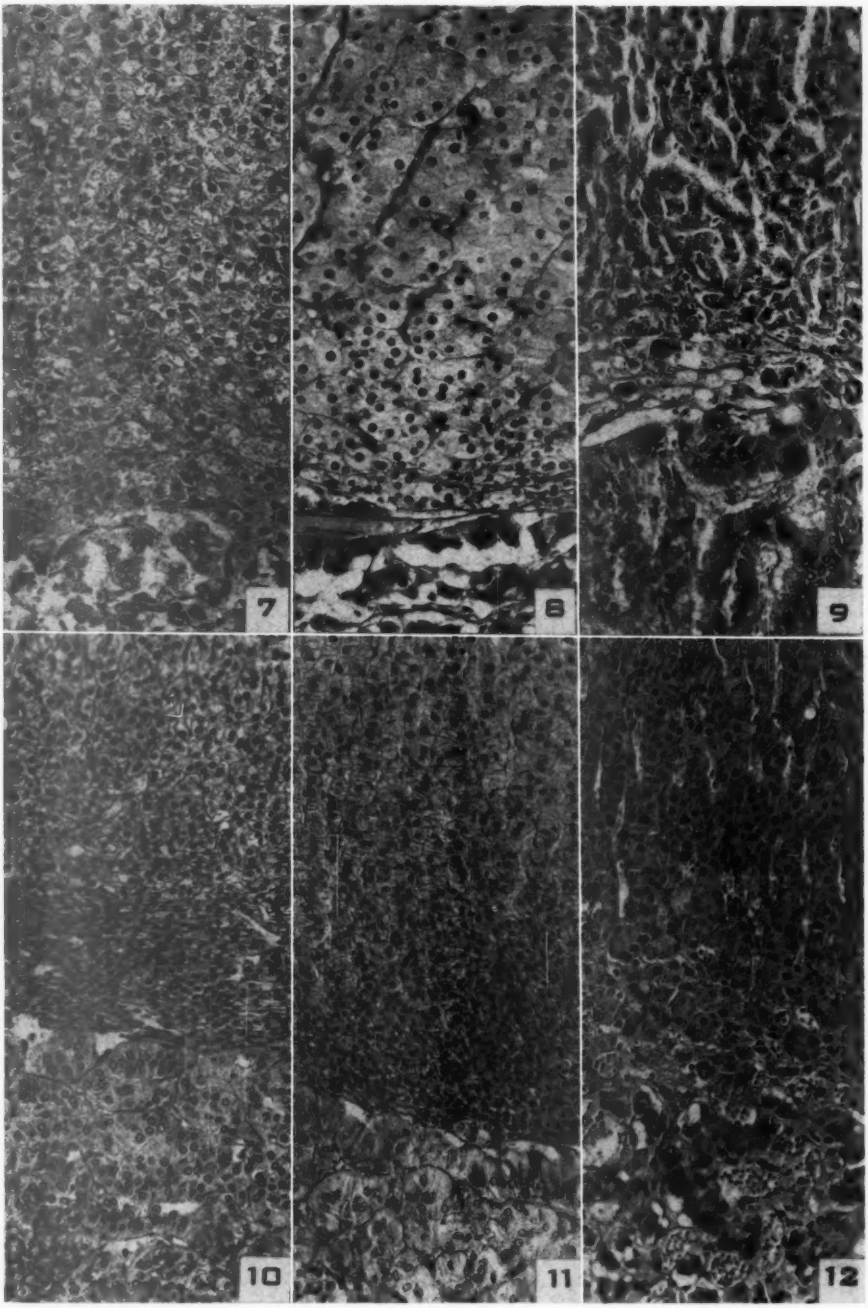
FIG. 8. Zonae fasciculata and reticularis and outer cells of medulla of adrenal gland shown in Figure 2. The appearances of the cortical cells in Figures 7 and 8 suggest that a considerable quantity of lipid remained in the adrenal glands of these animals. The cells of the medulla in Figures 7 and 8 were shrunken and fragmented. × 250.

FIG. 9. Zonae fasciculata and reticularis and outer cells of medulla of adrenal gland shown in Figure 3. Lipids apparently were completely removed from the cortical cells here, and some cells disappeared from the reticularis, showing the condensed stroma more clearly. × 125.

FIG. 10. Zonae fasciculata and reticularis and part of medulla of the adrenal gland shown in Figure 4. × 125.

FIG. 11. The inner zona fasciculata and the zona reticularis and part of the medulla shown in Figure 5. × 125.

FIG. 12. The inner zona fasciculata and the zona reticularis of the adrenal gland shown in Figure 6. Figure 10 illustrates cortical atrophy associated with necrosis of the pituitary body. Figure 11 illustrates cortical atrophy associated with inactivity, and Figure 12 allows comparison of cell sizes in these atrophic adrenal glands with the adrenal gland of an active animal.



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